Title:

Impact of genetic diversity of an earthworm on decomposition and ecosystem functioning

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ABSTRACT

Ecosystem functioning is affected positively by increased biodiversity, through complementary functions of multiple species or because high-functioning species are more likely in a species-rich community. Genetic diversity is one level of biodiversity that has been shown to positively affect ecosystem functioning. Whether the genetic diversity of a key decomposer species affects decomposition processes, and ecosystem functioning in general, is still unknown. We compared low and high genetic diversity assemblages of the earthworm *Dendrobaena octaedra* in two different experiments: using microcosms containing a simple community of other decomposer animals (some nematodes and other microfauna) and using microcosms with a more complex community, including also another earthworm species, diverse microarthropod fauna and enchytraeid worms. We studied the soil decomposition activity through measurements of mineral nitrogen and CO_2-production. In addition, the wellbeing of the earthworms was followed by determining their biomass, cocoon production, mortality and gene expression. Very few differences between low and high diversity microcosms were found, often in only one of the years studied. When differences were found, these depended on which genotype was present in the low diversity treatment. Our results suggest it is not genetic diversity *per se*, but specific genotypes of key decomposer species that can affect ecosystem functioning.

Keywords: intraspecific diversity, diversity-functioning relationship, *Dendrobaena octaedra,* soil food webs

Abbreviations: LD = low genetic diversity; HD = high genetic diversity; AkRed = aldo/keto reductase; ChymInh = chymotrypsin inhibitor; DualPhos = dual specifity phosphatase 14; GlucReg = glucose-regulated protein 94; PanAmy = pancreatic amylase; MT = metallothionein

1. INTRODUCTION

There is often a positive correlation between biodiversity and ecosystem functioning, referred to as the diversity-functioning relationship. In diverse communities, species can occupy complementary ecological niches and enhance resource use, and high-functioning species are more likely to be present [1,2,3,4,5]. Therefore, biodiversity is expected to have a positive impact on functional processes, such as primary production, decomposition and nutrient cycling, which affect energy flow in ecosystems.

Intraspecific genetic diversity is one component of biodiversity, and, like species diversity, it can have important effects on ecological processes. For example, high genetic diversity is positively associated with increased primary production and faster recovery from disturbances, via effects on interspecific competition and decomposition [6]. Studies that specifically address the effect of genetic diversity on ecosystem functioning have been focused on plants [7,8,9,10,11]. In a meta-analysis, Whitlock [12] found a positive correlation between intraspecific diversity and ecological functioning in plants, but the relationship was dependent on whether the focus was on adaptive genetic diversity (positive correlation) or neutral genetic diversity (positive correlation only under a limited set of demographic conditions). Effects of faunal genetic diversity on ecosystem processes are less studied, but higher genetic diversity has a positive effect on population parameters with consequences for ecological and evolutionary dynamics, e.g. in the marine invertebrate *Bugula neritina* [13] and in the black surfperch *Embiotica jacksoni* [14]. Recently, Des Roches and colleagues [15] and Raffard and colleagues [16] have shown through meta-analyses of both flora and fauna that intraspecific diversity (genotypic and/or phenotypic richness) impacts ecosystem processes as much as interspecific diversity. However, effects of intraspecific diversity on ecosystem function can vary among functional groups [16].

66 In soils, biodiversity affects functional biological processes, but the relative importance of
67 genetic, species and functional diversity in explaining the diversity-functioning relationship is still
68 unclear [17,18,19]. Some studies suggest that e.g. plant productivity and decomposition are
69 insensitive to loss of decomposer diversity, and that it is the presence of key-species and
70 functionally different organismal groups, rather than species diversity *per se*, that is important for
71 functional processes in soils [20,21,22,23], or that the effect of species diversity on ecosystem
72 functioning is inconsistent [24]. On the other hand, other studies show that species diversity, in
73 addition to functional differences and the presence of key-species, affects ecosystem functioning
74 positively [25,26,27,28]. Because there is a hierarchy of diversity levels within soils (functional,
75 species and genetic diversity), decomposition processes might be affected by functional diversity
76 more than by the genetic diversity of specific taxa, but genetic diversity might be particularly
77 important for specific processes, such as methane metabolism [29].

78 Hughes and colleagues [6] suggested that genetic diversity could be especially important
79 for ecosystem function in highly variable environments or when the environment is subjected to
80 anthropogenic changes. Anthropogenic contaminants can have negative impacts on organisms and
81 reduce species diversity [30,31,32]. For example, in soils, metal contamination can have both acute
82 and long-term biological effects, resulting in a negative impact and strong selective force on the
83 species diversity of earthworms [33,34,35]. However, even though species diversity can be as a
84 consequence of metal contamination in soils, genetic diversity of the species that are able to persist
85 in metal contaminated habitats is not necessarily affected. Metal contamination had no or only a
86 negligible effect on genetic diversity of the earthworm *Dendrobaena octaedra* [36,37,38], the
87 enchytraeid worm *Cognettia sphagnetorum* [39], the springtail *Orchesella cincta* [40] and
88 woodlice *Porcellionides sexfasciatus* [41]; and higher genetic diversity was found in populations

89 of the beetle *Staphylinus erythropterus* from metal contaminated sites than in those from reference
90 sites [42]. In light of these observations, when species diversity is diminished in metal
91 contaminated environments, the genetic diversity of the remaining species could become more
92 important than species diversity for ecosystem functioning.

93 In order to investigate whether the genetic diversity of a decomposer species has an effect
94 on ecosystem functioning, we established microcosm experiments simulating two growing seasons
95 or "years" in which we manipulated genetic diversity and species diversity. The focal species was
96 the epigeic earthworm *Dendrobaena octaedra,* a key decomposer in boreal coniferous forests [43].
97 *D. octaedra* reproduces through apomictic parthenogenesis [44,45], and offspring are clones of
98 their parent [46]. Nevertheless, it has high genetic diversity, in both pristine and metal
99 contaminated habitats [36,37,38]. Our microcosms contained *D. octaedra* with either low genetic
100 diversity (clonal specimens) or high genetic diversity (different genotypes). In one experiment, the
101 microcosms contained a simple community of other soil mesofauna (no other taxa were
102 intentionally added) and in the other experiment, the microcosms contained a more complex
103 community (another earthworm species, enchytraeids and microarthropods were added). CO_2
104 production was measured as a proxy for biological activity and NH_4-N concentration as a proxy
105 for nutrient mineralization. Cocoon production, growth and mortality of the earthworms were also
106 recorded as measures of earthworm wellbeing in the microcosms, and we also examined the
107 expression of five genes associated with metabolism and one gene indicating stress-response as a
108 proxy for earthworm activity. Assuming that higher genetic diversity has a positive effect on
109 ecosystem processes, we hypothesized that biological activity and nutrient mineralization would
110 be higher in microcosms containing a focal decomposer with high genetic diversity. However, for
111 the second experiment, we hypothesized that increased complexity of the decomposer community

112 would mask any differences in the measured variables between low and high genetic diversity of
113 the focal species, since more functional groups contributing to decomposition were present.
114
115 ## 2. MATERIALS AND METHODS

116 ### 2.1. Sampling the earthworms and establishing the cultures

117 *Dendrobaena octaedra* were collected from Harjavalta (South-West Finland, 61°18'50"N,
118 22°08'30"E). Soil at this site is contaminated with Cu (~823 mg kg^{-1}) and Zn (~474mg kg^{-1}) due
119 to historical emissions from a nearby smelter [47]. Because of its ability to tolerate metal
120 contamination [34,37,48,49,50,51], *D. octaedra* is the predominant earthworm species at this site,
121 and only rarely are other earthworm species encountered [47]. Earthworms were collected by hand
122 in September 2012 and transferred to the laboratory in buckets with soil from the sampling site.
123 Cultures were established in the laboratory, each from a single juvenile or sub-adult earthworm,
124 in glass jars (Ø 8 cm, with perforated lids) containing uncontaminated organic-rich soil and horse
125 manure for nutrition. All cultures were maintained at 15 °C and the soil was changed every few
126 months for two years. When needed, the cultures were transferred to larger plastic jars (Ø 13 cm,
127 with perforated lids) to avoid overcrowding. Because *D. octaedra* reproduces through apomictic
128 parthenogenesis, all offspring of the founder earthworm, and their offspring, are assumed to be
129 genetic clones, and each culture contained only one genotype [46].

130 ### 2.2. The experimental setup

131 Microcosms were established and the experiments were started in November 2014. We examined
132 the effect of *D. octaedra* genetic diversity on decomposition processes using two different
133 treatments: low diversity (LD; added *D. octaedra* were clonal specimens) and high diversity (HD;

added *D. octaedra* were different genotypes). In the low diversity treatments, four earthworms of the same genotype (from the same culture) were put into each microcosm. Three different cultures (clone families LD1-3) were used, and genotyping revealed that two of the clone families (LD2 and LD3) had the same multilocus genotype based on five microsatellite markers (See Supplementary Material 1 for the genotypes used as well as the procedure for DNA extraction and genotyping). Six (LD1) or four (each for LD2 and LD3) replicates of the low diversity treatments were established. In the high diversity treatments, four earthworms of different genotypes were put into each microcosm. Three genotype-compositions were used and there were four replicates of each (See Supplementary Material 1). In this experiment, the focal species was part of a simple decomposer community, meaning that no other taxa were intentionally added to the microcosms, but some taxa (nematodes and other microfauna and a few microarthropods) were transferred together with the soil and when the earthworms were introduced to the microcosms (See Supplementary Material 2).

A second experiment was prepared to examine whether the impact of a focal species' genetic diversity on decomposition is modified by the complexity (or species diversity) of the decomposer community. A similar set of treatments and replicates were prepared as described above, except one of the *D. octaedra* individuals was intentionally replaced with one *Dendrodrilus rubidus* earthworm. *D. rubidus* is another epigeic earthworm, with a slightly different feeding strategy than *D. octaedra* [52], allowing a possibility for increased impact on decomposition processes through complementation. In addition, we added ten individuals of the enchytraeid *Cognettia sphagnetorum* (previously extracted from organic rich soil using a standard wet funnel method) and samples of soil microarthropods (extracted from soil samples equivalent to the soil used in the microcosms). Differences in the abundance of other taxa (besides *D. octaedra*) between

157 the experiments were confirmed after each year, but for the first year, abundances of
158 unintentionally added taxa were high in both experiments (Supplementary Material 2).

159 For each experiment, there were 14 microcosms with the LD treatment and 12 microcosms
160 with the HD treatment (26 per experiment, total 52). Soil used in the experiments was collected
161 from an uncontaminated mature spruce forest in Jyväskylä and some fallen birch (*Betula pendula*)
162 leaves were shredded and mixed well with the soil for food (pH = 5.2; organic matter content =
163 79.5%). Water was added to the soil to achieve appropriate moisture, and during the experiments,
164 water was added to the microcosms about every other week (except during "winter") to replace
165 what was lost from evaporation. Microcosms were kept in a climate chamber with changing
166 temperature to mimic different seasons. The experiment was started with autumn, with temperature
167 first set to 12 ^{o}C and gradually decreasing to 1 ^{o}C over four weeks to winter. Winter lasted three
168 weeks with a steady temperature of 0.5 ^{o}C. Temperature increased in spring to 12 ^{o}C over three
169 weeks. Summer lasted 11 weeks, during which temperature increased to 13 and 14 ^{o}C, one week
170 each, and then was held at 15 ^{o}C for seven weeks before decreasing back to 14 and 13 ^{o}C, one
171 week each. After the first summer, half of the microcosms of each experiment were destructively
172 sampled and a second simulated year was continued with the remaining microcosms (following
173 the same temperature regime). Soil was replaced in those microcosms continuing in the second
174 year, and for the complex community experiment, new individuals of other decomposer animals
175 were added to the microcosms. Positions of the microcosms in the climate chamber were
176 randomized and varied after each data collection point (described below).

177 2.3. Collecting data

178 NH_4-N concentration was measured from the microcosms that were destructively sampled after
179 the first year and at the end of the experiment, as well as from the original soil: soil was extracted
180 with 2 M KCl-solution, and extracted samples were analyzed by Nablabs Oy (Jyväskylä) using
181 SFS-EN ISO 11732:2005 method. CO_2 production was measured throughout the experiment with
182 a Calanus UniQuant carbon analyzer, eight measurements during the growing season; twice in the
183 autumn and spring, and about every two weeks in the summer. Production of CO_2 was measured
184 by first determining the base level of CO_2 by injecting 1 ml of air from the microcosms to the
185 carbon analyzer, then closing the microcosm and keeping it air-tight for 1.5 h (during which the
186 microcosms were back in the climate chamber). Afterwards, another 1 ml sample of air was taken
187 and analyzed for CO_2. CO_2 production was calculated from the difference between the two samples
188 taking into account the air volume of each microcosm and the amount of soil ($\mu g\ g^{-1}\ h^{-1}$). Data
189 from all measurements (throughout the growing season) were analyzed together.

190 Biomass of the earthworms was measured at the beginning of the experiment, after the first
191 year, and at the end of the experiment. In the analyses, initial biomass was used as a co-variant.
192 Cocoon production (all cocoons, including cocoons of *D. rubidus* in the complex community
193 experiment), numbers of other decomposer animals, as well as mortality of *D. octaedra* were
194 recorded after the first year and at the end of the experiment.

195 Expression of five genes involved in metabolism and one gene involved in stress-response
196 was measured from *D. octaedra* after the first year and at the end of the experiment. Earthworms
197 were removed from the microcosms, and after weighing, put in glass jars with moist paper towel
198 for two days to allow them to empty their guts. Paper towel was changed after one day. A piece of
199 tissue (ca. 5 mm) from the anterior end of each earthworm was cut with a scalpel, placed in separate
200 1.5 ml microcentrifuge tubes and immediately frozen in liquid nitrogen. Samples were stored at -

80 °C until RNA extraction. Gene expression was measured from aldo/keto reductase (AkRed), chymotrypsin inhibitor (ChymInh), dual specifity phosphatase 14 (DualPhos), glucose-regulated protein 94 (GlucReg), pancreatic amylase (PanAmy) and metallothionein (MT). Expression of 18S and 28S ribosomal RNA genes was measured to be used as reference genes in normalization. All primers, except those for MT, 18S and 28S [47], were designed based on a draft transcriptome of *D. octaedra,* in which gene annotations and GO-terms were assigned to sequences (shared by M. Holmstrup, unpublished, Supplementary Material 3).

2.4. RNA extraction and qPCR protocol

For RNA extraction, we used the Aurum Total RNA mini kit (Bio-Rad) following the manufacturer's protocol including DNase I treatment. Concentration of the extracted RNA was measured using the Qubit RNA Assay Kit and Qubit Fluorometer (Invitrogen, Turner BioSystems). For cDNA synthesis, we used the iScript cDNA synthesis kit (Bio-Rad) following the manufacturer's protocol, using 35 ng of RNA per reaction. After synthesis, cDNA was diluted 1:5. Real-time quantitative polymerase chain reactions (qPCR) were performed using IQ SYBR green supermix (Bio-Rad). qPCR conditions were optimized and efficiencies were checked prior to the study with a dilution series (5 points, 10-fold dilutions) (Supplementary Material 3). In each reaction we used 1 µl of cDNA template and 0.5 µM of each primer in a final reaction volume of 20 µl (for the efficiency estimates) or 10 µl (for the gene expression measurement). Three replicate reactions for each sample were prepared and an inter-run calibrator was used. For all reactions, we used a CFX96 C1000 Touch Thermal Cycler (Bio-Rad) with the following protocol: 94°C for 2 minutes and then 40 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 15 sec followed by a plate read. A melt curve analysis was done at the end of each amplification reaction to ensure a single amplification product.

224 2.5. Data analysis

225 Raw gene expression data and stability of the reference genes were checked with qBase+
226 (Biogazelle). 18S and 28S could not be used as reference genes for this dataset, and so NORMA-
227 Gene [53] was used for normalization. NH_4-N concentration, CO_2 production, cocoon production,
228 earthworm biomass, mortality and the normalized gene expression data were analyzed in IBM
229 Statistics SPSS version 20. Conformity to a normal distribution was tested using a Kolmogorov-
230 Smirnov test or Shapiro-Wilk test when appropriate, and homogeneity of variances was examined
231 with Levene's test. Data were log-transformed when necessary to meet those assumptions, but it
232 did not help in all cases. Our main focus was to test the differences between low (LD) and high
233 genetic diversity (HD) treatments, which was tested in general (combining data from both
234 experiments irregardless of the diversity of other decomposer taxa) and also separately for the
235 different experiments (simple or complex decomposer communities) for both the first and the
236 second year of the experiment. Differences between the LD microcosms were also tested, and
237 since there were only two genotypes in the LD treatments (LD1 had one genotype while LD2 and
238 LD3 had another), the difference between LD and HD was also tested for the two genotypes
239 separately. Analysis of the data was restricted to simple one-way ANOVA or Kruskal-Wallis tests,
240 since the relatively small sample size of our study could lead to very high mean errors in more
241 complex analyses (e.g. when including interactions).

242 3. RESULTS

243 3.1. Soil mineral nitrogen

244 There was no difference between low (LD) and high genetic diversity (HD) treatments in soil NH_4-
245 N concentration in general and when analyzing the experiments with simple and complex

246 communities of other decomposers separately (Table 1, Figure 1). When comparing the LD
247 genotypes, soil NH_4-N concentration was higher in microcosms with LD1 than it was in
248 microcosms with LD2 and LD3 (Table 2, Supplementary Material 1). We also compared the HD
249 treatment to the two genotypes in LD separately: when comparing HD to LD1, NH_4-N
250 concentration was higher in LD than HD, but when comparing HD to LD2 and 3, there was no
251 difference (Table 2 and Supplementary Material 1).

252 3.2. CO_2 production

253 There was no difference in CO_2 production between LD and HD treatments in general and when
254 analyzing the different years of the experiment separately (Table 1, Figure 2; mean in LD: 3.44
255 µg/g/h, mean in HD: 3.33 µg/g/h). However, when analyzing the experiments with simple or
256 complex decomposer communities separately, there was less CO_2 produced in the HD treatment
257 than in LD in the presence of a complex community in the first year (Table 2, Figure 2). There
258 was no difference between the LD families, and when comparing HD to the two LD genotypes
259 separately (Table 2).

260 3.3. *D. octaedra* growth and cocoon production

261 In general, there were no differences in the biomasses of the earthworms between LD and HD
262 treatments (Table 1, Supplementary Material 2). However, in the second year, earthworms from
263 LD1 had higher biomass than those from LD2 (LSD: mean difference: 0.060, $p<0.001$) and LD3
264 (LSD: mean difference 0.061, $p<0.001$) (Table 2, Supplementary Material 1). When comparing
265 HD to LD1, there were no differences in earthworm biomass in either year, but when comparing
266 HD to LD2 and LD3, there was higher earthworm biomass in HD in the second year (Table 2,
267 Supplementary Material 1).

There were no differences in cocoon production between LD or HD treatments, both in general and when the experiments of simple and complex decomposer communities were analyzed separately (Table 1). More cocoons were produced in microcosms with LD1 than those with LD2 or LD3 (Table 2, Supplementary Material 1). When comparing HD to LD1, there was higher cocoon production in LD1, but there was no difference in cocoon production when comparing HD to LD2 and LD3 (Table 2).

3.4. Mortality and other species

Mortality of *D. octaedra* differed between LD and HD treatments in the first year. Specifically, there was higher mortality in HD treatments in the experiment with a simple community of other decomposer animals (Table 1). Mortality did not differ between different LD families (Table 2). However, mortality was higher in HD than in LD1 (Table 2; HD mortality 17, LD1 mortality 1). In the experiment with a complex community of other decomposer animals, mortality of *D. rubidus* was high in all microcosms, with only a few individuals found alive after the first year and at the end of the experiment.

3.5. Gene expression

In general, gene expression differed between LD and HD treatments in only one of the genes: expression of ChymInh was higher in HD in the second year (Table 1, Figure 3). Examining the experiments with simple and complex decomposer communities separately, earthworms in HD treatments showed higher expression of ChymInh in both years when there was a simple decomposer community (Table 1, Supplementary Material 2). Expression of DualPhos was higher in earthworms in the LD treatments in the second year when there was a complex decomposer community, and expression of MT was higher in earthworms in the HD treatments in the first year

290 (Table 1, Supplementary Material 2). Comparing the different clone families, expression of AkRed
291 was higher in LD1 than in LD2 (Post hoc LSD: mean difference 0.434, p = 0.043) and LD3 (Post
292 hoc LSD: mean difference 0.453, p = 0.032). When comparing HD to LD2 and LD3, there were
293 no differences in gene expression, but when comparing HD to LD1 there was higher expression of
294 AkRed in LD, and higher expression of ChymInh in HD (Table 2, Supplementary Material 1).

295 4. DISCUSSION

296 Intraspecific diversity has a positive effect on ecosystem functioning, as does species diversity
297 [15,16]. To our knowledge, however, the functional effect of intraspecific genetic diversity of a
298 key species on decomposition processes has not been studied before, even though decomposer
299 animals are integral components of terrestrial ecosystems. Using microcosm experiments, we
300 investigated whether higher genetic diversity in the earthworm *Dendrobaena octaedra* leads to
301 higher decomposition activity than does lower genetic diversity. We also wanted to investigate
302 what effect functional and species diversity of the decomposer community might have on the
303 relationship between genetic diversity of *D. octaedra* and decomposition activity. Therefore, in
304 one of our experiments the earthworms were part of a complex decomposer community, while in
305 the other, they were part of a simple decomposer community.

306 Overall, very few differences were found between low and high genetic diversity (LD vs.
307 HD) microcosms: there were no differences in mineral nitrogen and CO2-production, but *D.*
308 *octaedra* had higher mortality and higher expression of ChymInh in HD treatments compared to
309 in LD treatments. So, our hypothesis that high genetic diversity would have a positive effect on
310 decomposition processes was not supported by the experiment. However, when looking at the
311 microcosms with simple or complex decomposer communities separately, we found other

differences between LD and HD treatments. Specifically, when a complex community was present, there was lower CO_2 production as well as lower expression of DualPhos and higher expression of MT in microcosms with high genetic diversity. We hypothesized that the presence of a complex decomposer community might prevent us from detecting differences in decomposition activity between LD and HD treatments because there are more functional groups present when there are more species in the community. In our experiment the additional functional groups seemed to have a negative effect on the worms in HD treatments. For the most part, the few differences between treatments that we observed were apparent only in one of the two years studied. The only exception was expression of ChymInh in the microcosms with a simple community, being higher in HD in both years. The results suggest that genetic diversity of the focal decomposer species does not have a strong impact on decomposition activity. Our results support the proposition of Kardol and colleagues [29] that genetic diversity of members of soil food webs will have only minor effects on ecosystem level processes.

Higher mortality in HD treatments than in LD treatments, observed particularly in the first year of the experiment, could indicate a possible technical artifact or a genotype specific phenomenon and might not necessarily be a result of the genetic diversity treatments. In the experiment with a complex decomposer community, the HD treatments included the focal species *D. octaedra* as well as one individual of another epigeic earthworm, *Dendrodrilus rubidus.* High mortality of *D. rubidus* could have had a negative impact on *D. octaedra,* as the *D. rubidus* decomposed within the microcosms. Also, since many more earthworms were needed per genotype in the LD treatments to create the needed replicates for the experiments, the clone families chosen were those that produced abundant offspring, which could indicate higher fitness than the genotypes used in creating the HD treatments. Indeed, a difference in mortality was

observed when HD was compared with LD1, but not when HD was compared to LD2 and LD3. Alternatively, higher mortality in HD might indicate some competition between different genotypes that did not occur among individuals in LD treatments.

Expression of the gene putatively coding for the protein chymotrypsin inhibitor (ChymInh) was higher in HD overall (in the second year) and higher in HD microcosms in the experiment with a simple decomposer community in both years, which might suggest some effect of genetic diversity on decomposition that could have an impact in the long-term. Chymotrypsin inhibitor inhibits the production of chymotrypsin, a digestive enzyme involved in protein degradation [54], but other functions also have been described for this protein, e.g. involvement in the immune response [55]. Because the specific functions of this and the other gene products studied have not been experimentally confirmed in *D. octaedra*, we relied on GO-terms originally assigned to the transcriptome sequences, and we can only speculate about functional responses resulting from gene expression. Whether the expression of ChymInh has a specific effect on decomposition is not clear, but as a proxy for metabolic activity, it indicates that *D. octaedra* in the HD microcosms had higher metabolic activity than those in LD microcosms, which might translate into functional differences. But, it must be noted that there were few differences between HD and LD treatments in expression of the other genes linked to metabolism. Higher expression of ChymInh in HD could also indicate a stress response of the worms, which would be in line with our observation of increased mortality of *D. octaedra* in the HD treatments. The fact that differences in expression of this gene between the diversity treatments were not observed when there was a complex community present indicate that, at least for this measure, complementary effects from interactions with other species could possibly mask differences between populations with different levels of diversity. In contrast, expression of metallothionein (MT, described in [47]), a gene involved in

stress-response, showed expression differences between LD and HD treatments only when a complex decomposer community was present.

Our experiments revealed that the specific genotype or lineage of *D. octaedra* seems to have a greater impact on decomposition processes than does genetic diversity *per se.* Even when there were no differences between diversity treatments overall (in nitrogen mineralization, earthworm biomass, cocoon production and gene expression) there were differences between the LD treatments, with the LD1 genotype being significantly different from the LD2 and LD3 genotype. And for most of the measured variables (all except earthworm biomass), LD1 treatments also differed from HD treatments. Interestingly, the genotype used in LD1 was from the most productive culture among all of our cultures, indicating high fitness under laboratory conditions, and presumably also in the natural environment.

5. CONCLUSIONS

We hypothesized that higher genetic diversity of a key decomposer animal species could be beneficial for the functioning of terrestrial ecosystems through more efficient decomposition processes, but results from our microcosm experiments did not support this hypothesis. Nevertheless, it seems that particular genotypes could prevent significant changes in ecosystem functioning even when species and genetic diversity has been reduced by anthropogenic effects, e.g. metal contamination of soil. Anthropogenic contaminants can reduce species diversity [30,31,32], and in these circumstances, ecosystem functioning depends on the remaining species and genotypes. Higher standing genetic diversity makes it more likely that some genotypes are able to tolerate well the changed environmental conditions, and provides a means for continued ecosystem functioning despite declines in biodiversity.

Competing Interests

The authors declare no competing interests.

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Data Statement

Data collected for this manuscript are published in the Jyväskylä University Digital Repository (JYX accession number to be added after acceptance for publication).

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525

526 **Figure 1**. Concentration of NH_4-N (µg/g dry mass) in low diversity (LD; dark grey) and high diversity
527 (HD; light grey) treatments in the presence of a simple or complex community of the other decomposer
528 animals. Data from the first and second years of the experiment are shown separately.

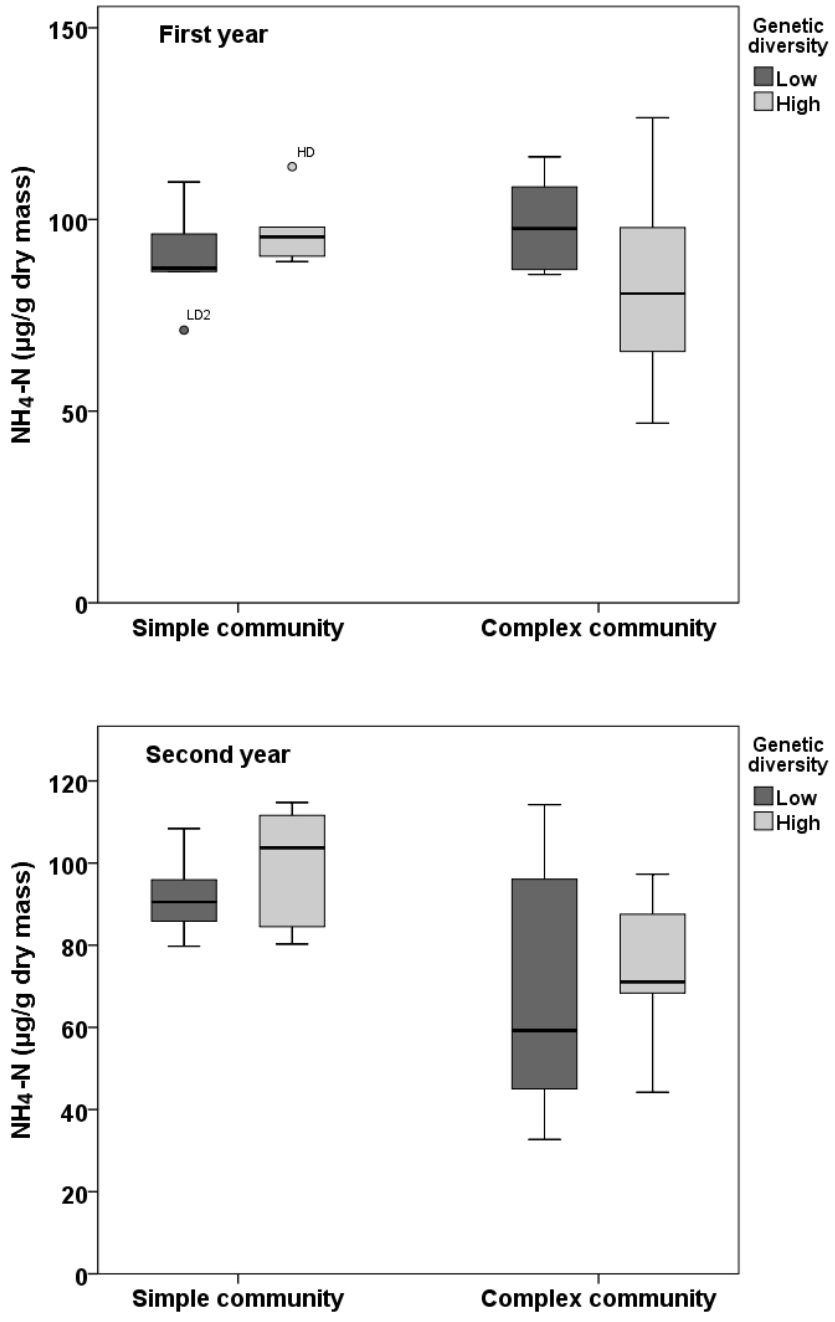

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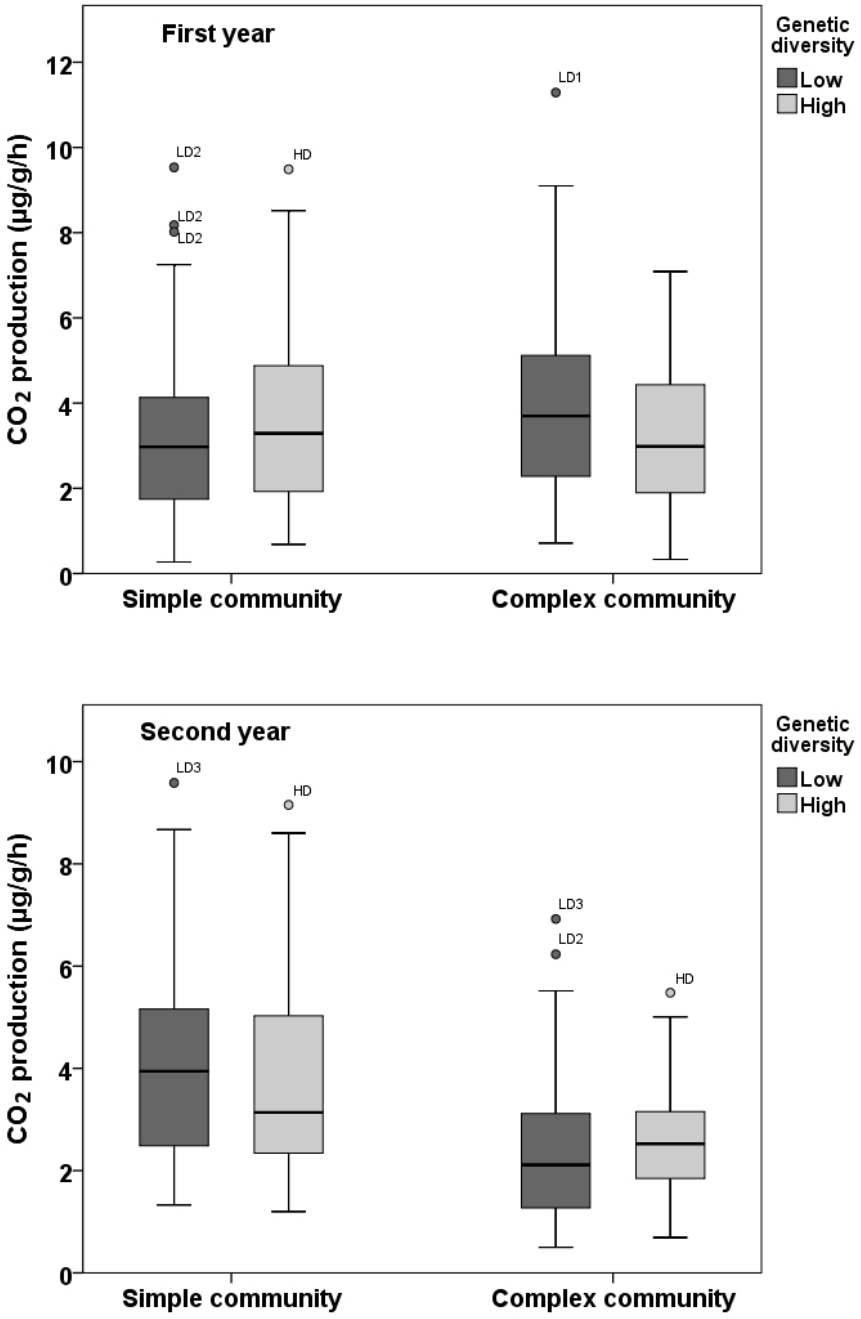
Figure 2. CO_2 production (µg/g/h; means of all measurements done throughout the experiment) in low diversity (LD; dark grey) and high diversity (HD; light grey) treatments in the presence of a simple or complex community of the other decomposer animals. Data from the first and second years of the experiment are shown separately.



535 **Figure 3**. Gene expression (Normalized Relative Quantity) in low diversity (LD; dark grey) and high
536 diversity (HD; light grey) treatments in the presence of a simple or complex community of the other
537 decomposer animals. Data from the first and second years of the experiment are shown separately.

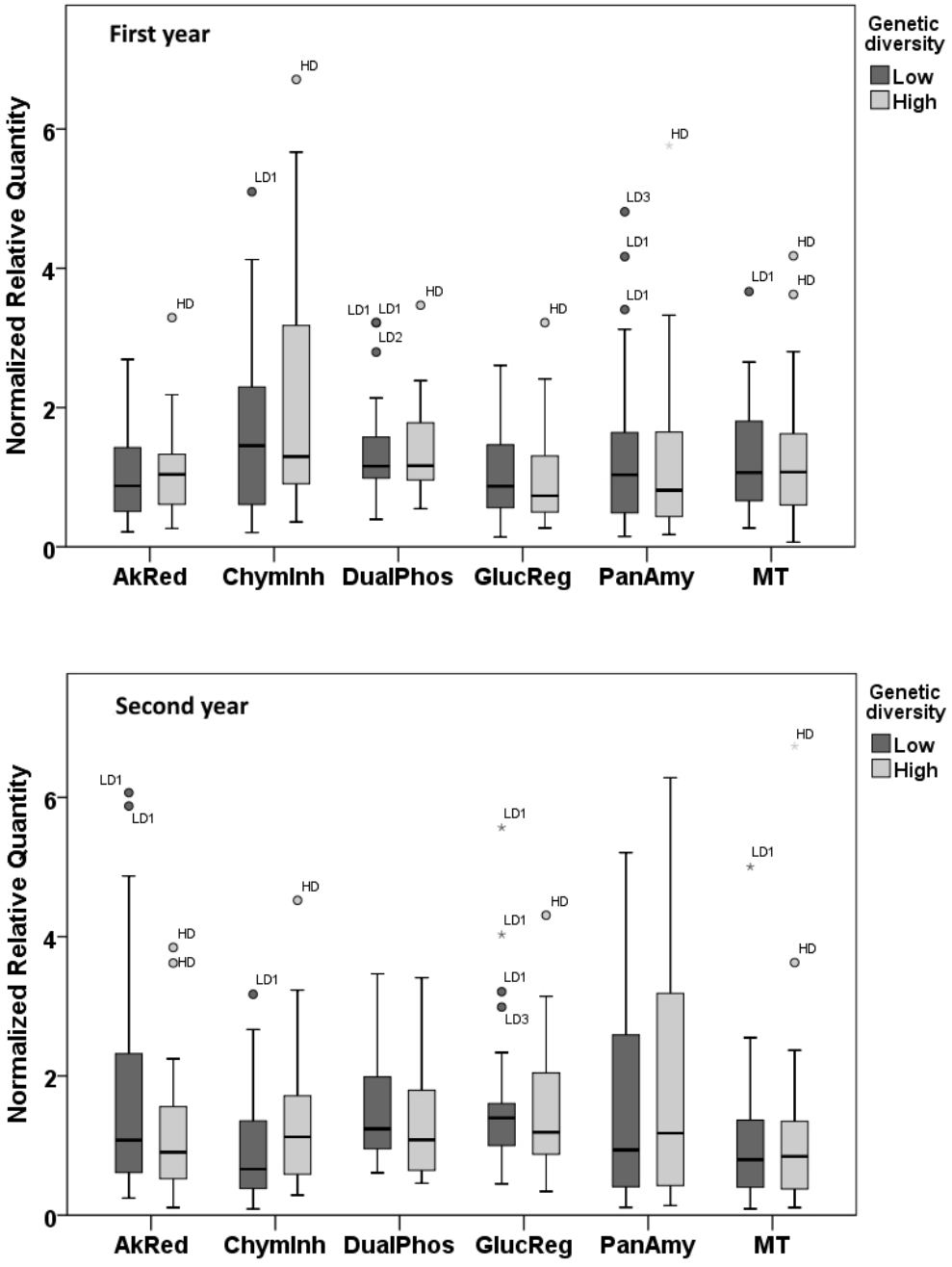


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Table 1. Differences between low diversity (LD) and high diversity (HD) treatments in measured parameters with the first and second growing seasons separated. NH_4- concentration, CO_2 production and cocoon production were analyzed with Kruskal-Wallis test with df = 1. Earthworm biomass was analyzed with one-way ANOVA, with biomass measured at the beginning of the experiment as a covariant and df = 1. "General" describes the general result, comparing all LD and HD microcosms (N = 28 and 24, respectively). "Simple" describes the comparison made only with microcosms from the experiment with the simple decomposer community and "Complex" the comparison made only with microcosms from the experiment with the complex decomposer community (in each, N = 14 and 12).

		First year		Second year	
NH_4-N concentration		X^2	p	X^2	p
	General	0.130	0.719	0.130	0.719
	Simple	1.653	0.199	1.306	0.253
	Complex	2.041	0.153	0.082	0.775
CO_2 production		X^2	p	X^2	p
	General	0.497	0.481	0.002	0.964
	Simple	2.677	0.102	0.919	0.338
	Complex	7.181	**0.007****	2.255	0.133
Earthworm biomass		F	p	F	p
	General	0.010	0.920	2.678	0.106
	Simple	0.002	0.966	1.316	0.257
	Complex	0.054	0.818	0.920	0.346
Cocoon production		X^2	p	X^2	p
	General	0.316	0.574	0.450	0.502
	Simple	0.096	0.756	0.021	0.886
	Complex	0.417	0.519	0.185	0.667
Earthworm mortality		X^2	p	X^2	p
	General	5.418	**0.020***	0.035	0.853
	Simple	4.172	**0.041***	1.167	0.280
	Complex	1.583	0.208	1.221	0.269
Gene Expression		F	p	F	p
AkRed	General	0.394	0.532	2.331	0.132
	Simple	3.560	0.068	0.016	0.899
	Complex	2.219	0.148	2.164	0.153

ChymInh	General	1.345	0.250	4.664	**0.035***
	Simple	8.577	**0.006****	6.097	**0.019***
	Complex	2.079	0.161	0.006	0.939
DualPhos	General	0.068	0.794	1.673	0.200
	Simple	0.806	0.375	0.114	0.737
	Complex	0.907	0.349	4.906	**0.035***
GlucReg	General	0.198	0.657	0.526	0.471
	Simple	0.095	0.760	2.194	0.147
	Complex	0.453	0.506	0.537	0.470
PanAmy	General	0.120	0.730	0.186	0.668
	Simple	3.720	0.062	1.300	0.262
	Complex	2.787	0.106	0.187	0.669
MT	General	0.482	0.490	0.000	0.998
	Simple	2.335	0.135	0.102	0.751
	Complex	8.994	**0.006****	0.320	0.576

548

549 **Table 2**. Comparisons between the different low diversity (LD) clone families, between high diversity
550 (HD) and the two low diversity genotypes separately (LD1 is one genotype, and LD2&LD3 are another).
551 Analyses were done either with Kruskal-Wallis test (X^2) or one-way ANOVA (F): NH_4-N concentration
552 (X^2), CO_2 production (X^2), Biomass (in the 1st and 2nd year, both using initial weight as a covariate (F),
553 cocoon production (X^2), mortality (X^2) and gene expression (F).

	Comparison between					
	LD families		HD vs. LD1		HD vs. LD2&LD3	
	X^2/F	p	X^2/F	p	X^2/F	p
NH_4-N	9.720	**0.008**	4.470	**0.035**	2.223	0.136
CO_2	4.867	0.088	0.121	0.727	0.489	0.484
Biomass						
1st year	0.654	0.522	0.171	0.680	0.253	0.616
2nd year	16.495	**<0.001**	0.743	0.393	13.300	**0.001**
Cocoons	8.370	**0.015**	6.286	**0.012**	0.507	0.477
Mortality	3.242	0.198	5.931	**0.015**	0.421	0.517
Gene Exp.						
AkRed	3.320	**0.042**	4.867	**0.030**	0.417	0.520
ChymInh	2.923	0.060	10.114	**0.002**	0.266	0.607
DualPhos	2.670	0.076	3.065	0.083	0.075	0.785
GlucReg	0.805	0.451	0.944	0.334	0.000	0.985
PanAmy	2.504	0.089	1.768	0.187	1.052	0.307
MT	0.024	0.976	0.160	0.690	0.255	0.614

554

555 **Supplementary Material:**

556 **Supplementary Material 1.** Genotypes of *Dendrobaena octaedra* earthworms used in the microcosm
557 experiments and comparisons between genotypes.

558 **Supplementary Material 2.** Comparisons of taxa in the microcosm experiments with simple and complex
559 decomposer communities.

560 **Supplementary Material 3**. Table of the primer sequences used in qPCR and reaction efficiencies.

561

Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supplement to the manuscript: Impact of genetic diversity of an earthworm on decomposition and ecosystem functioning by Mustonen, M., Haimi, J. Knott, K.E.

Supplementary Material 1. Genotypes of *Dendrobaena octaedra* earthworms used in the microcosm experiment and comparisons between genotypes.

Genotype diversity treatments

Low genetic diversity treatments LD1, LD2 and LD3 were made up of worms from clone families H1, H2, and H3, respectively. For microcosms with a simple decomposer community, there were four individuals from the respective clone families in each microcosm. For microcosms with a complex decomposer community, there were three individuals from the respective clone families in each microcosm plus one individual of *Dendrodrilus rubidus*. High genetic diversity treatments contained earthworms with differing genotypes, with either four or three individuals depending on whether the microcosm had a simple or complex decomposer community. Each microcosm of HD1 was composed of genotypes H1/H4/H8/(H6), each microcosm of HD2 was composed of genotypes H1/H8/H9/(H5), and each microcosm of HD3 was composed of genotypes H1/H2/H7/(H10). The genotype of the worm replaced by *Dendrodrilus rubidus* in the microcosms with a complex decomposer community is marked in parentheses.

Clone families H2 and H3 are the same genotype based on five microsatellite markers (DO1, DO2, DO3, DO4, DO6) (Mustonen et al. 2017).

Table 1. Genotypes (allele sizes) of *Dendrobaena octaedra* from the different clone families at five microsatellite markers (DO1, DO2, DO3, DO4, DO6). Note that because *D. octaedra* are hexaploid, there is a maximum of six alleles per locus. Each clone family was originated with a single juvenile or sub-adult individual, and all progeny are expected to be clones.

clone family	DO1	DO2	DO3	DO4	DO6
H1	231/237	140/153	165/173/177	211/213	96/100
H2	231	140	173/181/189	211/213	96
H3	231	140	173/181/189	211/213	96
H4	231/237	138/140	165/177/185/200	211/213	90/96
H5	229/231/233/235/237	131/138/143/145	165/167/171/177	211/213	90/96/100
H6	229/231/233/235/237	138/145/159	165/177/181/187/196	211/213	96/100
H7	227/229/231/233/235/237	138/145	165/173/177	211/213	100
H8	227/229/231/233/235/241	140	165/177/185	211/213	96
H9	229/231/233/241/245/253	140/147/151	177/185/187/193	211/213	90/96
H10	229/231/233/235/237	138/145	165/173/177	211/213	96/100

DNA extraction and genotyping

Genomic DNA was extracted from piece of the anterior end of the earthworm using Qiagen chemistry (DNeasy kit reagents) and a Kingfisher magnetic processor (Thermo Fisher Scientific). Five microsatellite loci were used for genotyping, DO1, DO2, DO3, DO4 and DO6 (described in Mustonen et al. 2017). Amplification was performed separately for each locus in 10 µl reactions

containing 1 µl of template DNA, 1X buffer (Biotools), 0.4 mM dNTPs (Fermentas), 1 µM reverse primer, 0.9 µM forward primer (TAG Copenhagen), 0.1 µM labeled forward primer (Applied Biosystems), 2 mM MgCl2 (Biotools) and 0.5 units Taq polymerase (Biotools). Conditions for thermocycling were 94 °C for 3 min, then 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, ending with a final extension of 72 °C for 10 min. Amplification products were separated using the ABI PRISM 3130xl with GeneScan 500 LIZ size standard and genotyped using GeneMapper 5 software (all Applied Biosystems).

Comparisons among genotypes

In addition to comparing the effects of genetic diversity (LD or HD treatments) on decomposition processes and activity in the experimental microcosms, we also compared our measured parameters among the different genotypes. Results of these comparisons are presented in the main text, and visualized below. In these comparisons, data from the two experiments are combined.

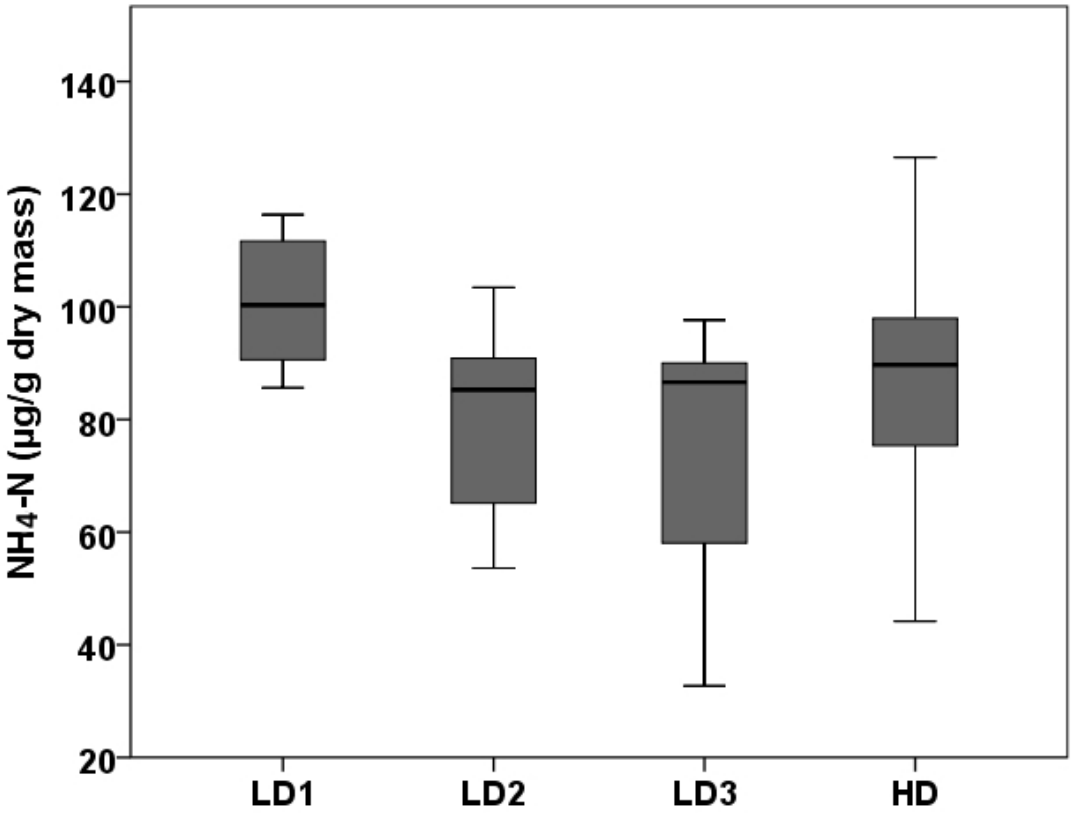

Figure 1. Concentrations of NH_4-H in the presence of the different low diversity families (LD1, LD2 and LD3) and the high diversity treatment (HD).

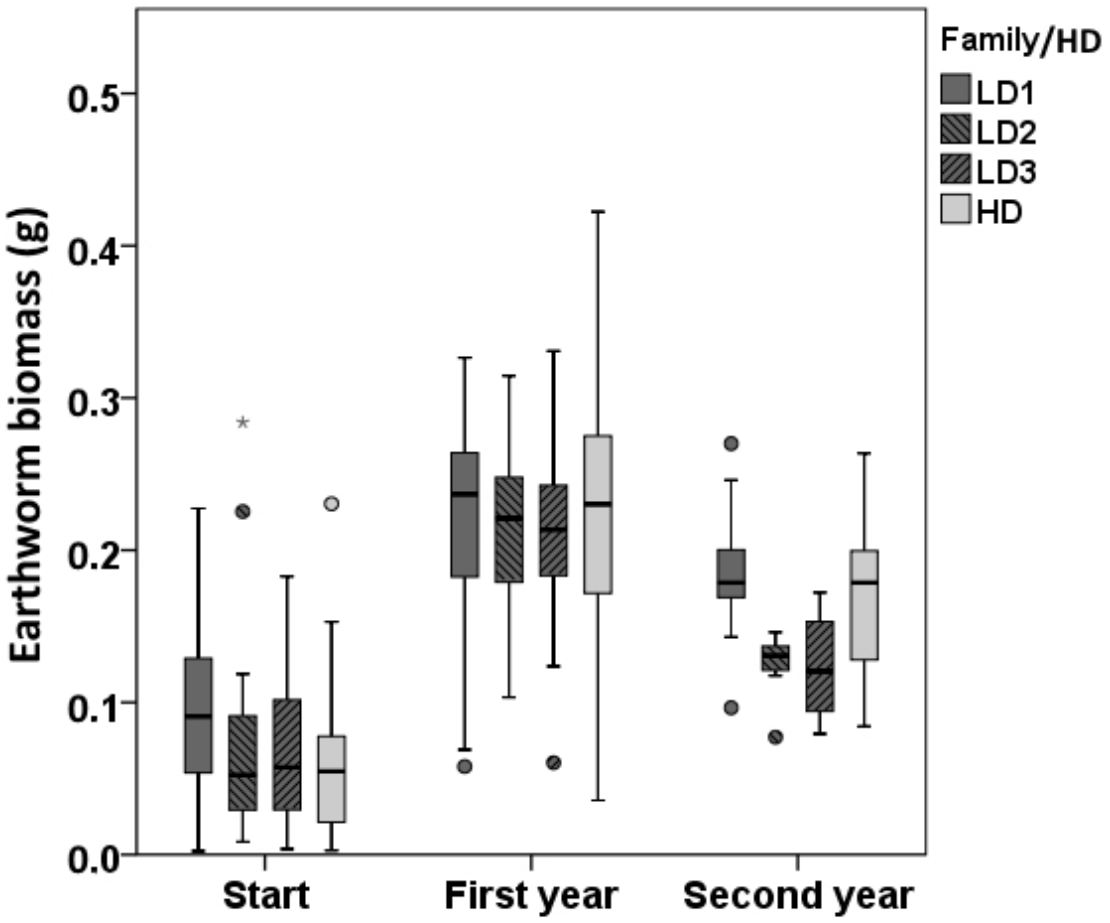

Figure 2. Biomass (g) of the earthworms in the different low diversity families (LD1, LD2 and LD3) and the high diversity treatment (HD) at the start of the experiment, after the first year and after the second year.

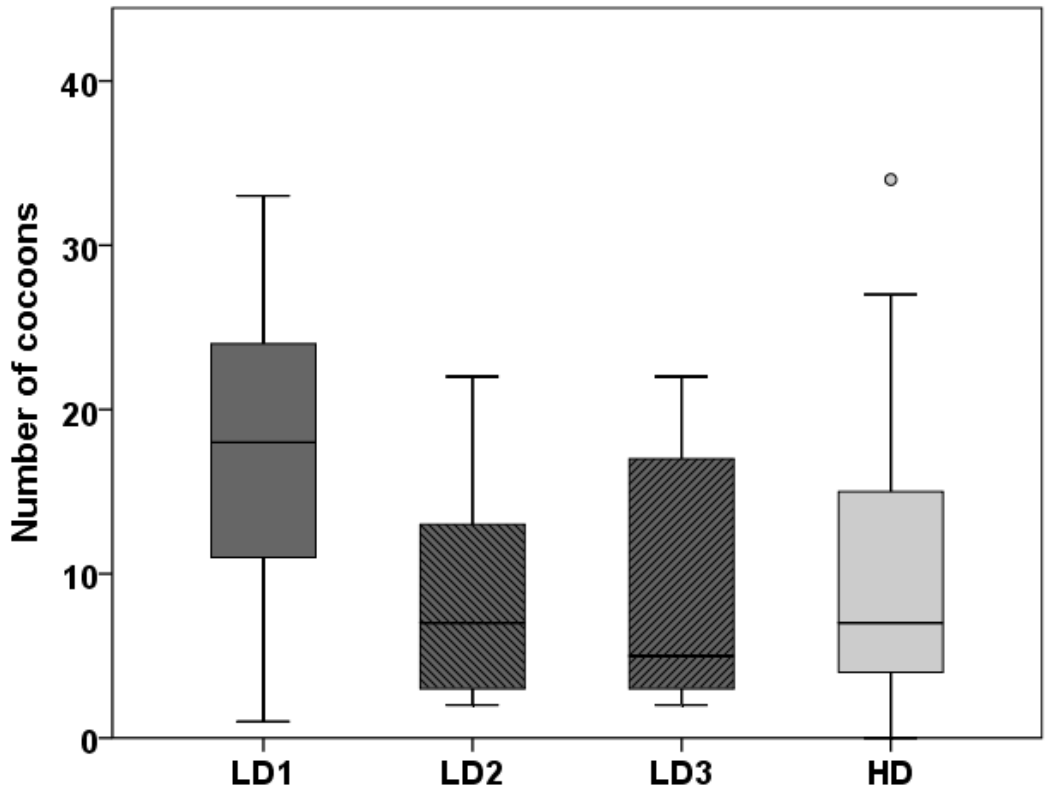

Figure 3. Number of cocoons produced by different low diversity families (LD1, LD2 and LD3) and high diversity treatment (HD).

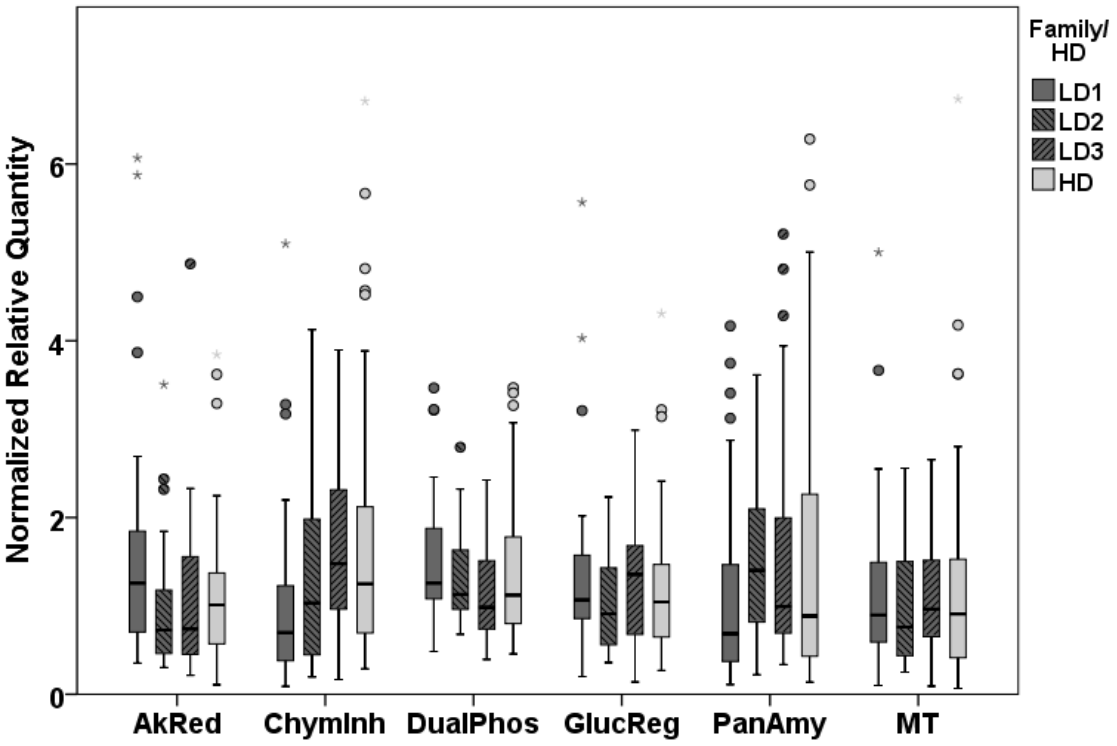

Figure 4. Gene expression (Normalized Relative Quantity) in the different low diversity families (LD1, LD2 and LD3) and high diversity treatment (HD).

Supplement to the manuscript: Impact of genetic diversity of an earthworm on decomposition and ecosystem functioning by Mustonen, M., Haimi, J. Knott, K.E.

Supplementary Material 2. Comparisons of taxa in the microcosm experiments with simple and complex decomposer communities.

Simple decomposer communities were constructed by adding only the focal decomposer species, *Dendrobaena octaedra,* to the experimental microcosms. For a complex decomposer community, we replaced one *D. octaedra* individual with another epigeic earthworm, *Denrodrilus rubidus,* and we added ten individuals of the enchytraeid *Cognettia sphagnetorum* (previously extracted from organic rich soil using a standard wet funnel method), as well as samples of soil microarthropods (extracted from soil samples equivalent to the soil used in the microcosms). Together with the added taxa, associated microfauna (nematodes, tardigrades and rotifers) were also included, even in the simple decomposer communities.

Table 1. Mean (SD) individuals of each additional decomposer animal group in the experiments with simple and complex community and LD and HD treatments (per microcosm), both after the first year and the second year.

1.year							2.year				
	Simple		Complex					Simple		Complex	
	LD	HD	LD	HD				LD	HD	LD	HD
Nematodes	133.5 (67.34)	133.25 (38.66)	89.79 (39.78)	65 (50.79)			Nematodes	187.94 (75.87)	69.5 (40.67)	538.29 (744.74)	28.5 (34.56)
Enchydraeids	0 (0)	0 (0)	139.93 (52.36)	74.5 (85.85)			Enchydraeids	0 (0)	0 (0)	11.14 (14.57)	12.5 (19.57)
Tardigrada	7.93 (6.72)	15.25 (12.03)	1.93 (2.53)	1.25 (2.70)			Tardigrada	0 (0)	0 (0)	0 (0)	0.5 (1.22)
Rotatoria	0.86 (2.48)	2.25 (3.17)	1.29 (2.27)	0.5 (1.17)			Rotatoria	0.431 (0.80)	0 (0)	0.43 (1.13)	0.5 (1.22)
Collembola	9.64 (10.20)	8 (14.82)	4.29 (13.50)	0 (0)			Collembola	0.43 (1.74)	0 (0)	1.29 (2.36)	1 (1.55)
Mesotigmata	0.64 (1.28)	0 (0)	5.36 (5.29)	3 (3.84)			Mesotigmata	0 (0)	0 (0)	6 (7.55)	3.5 (3.51)
Oribatida	4.93 (4.34)	2.75 (2.99)	15.86 (9.45)	11.75 (12.17)			Oribatida	1.71 (2.23)	0.5 (1.22)	10.29 (11.21)	4.5 (5.61)

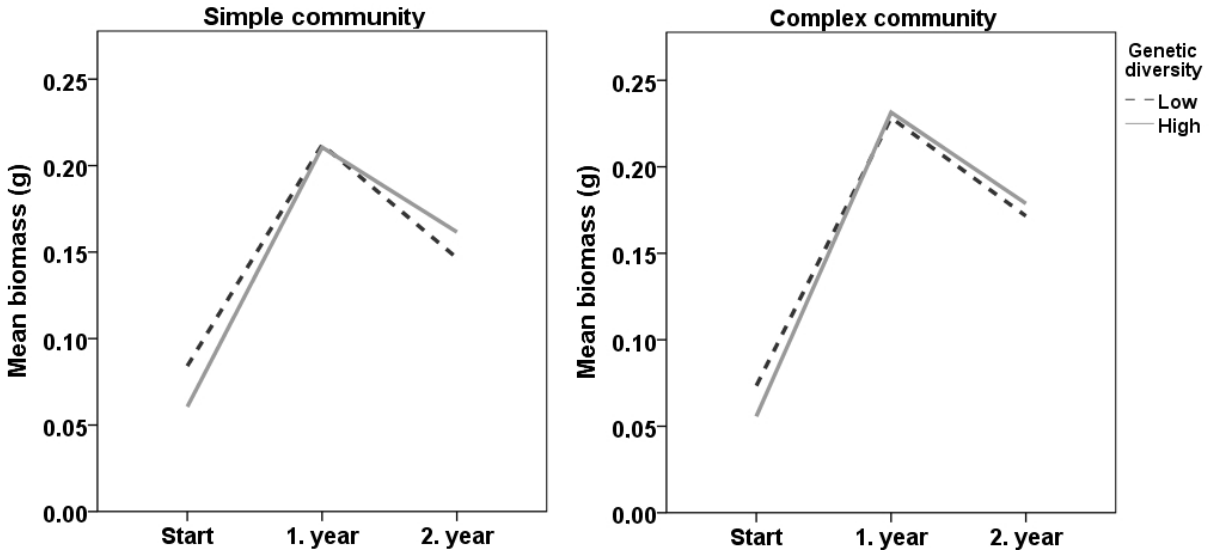

Figure 1. Biomass (g) of the earthworms in low diversity and high diversity treatments at the start of the experiments, after the first year and after the second year in the presence of a simple or complex community of other decomposer animals.

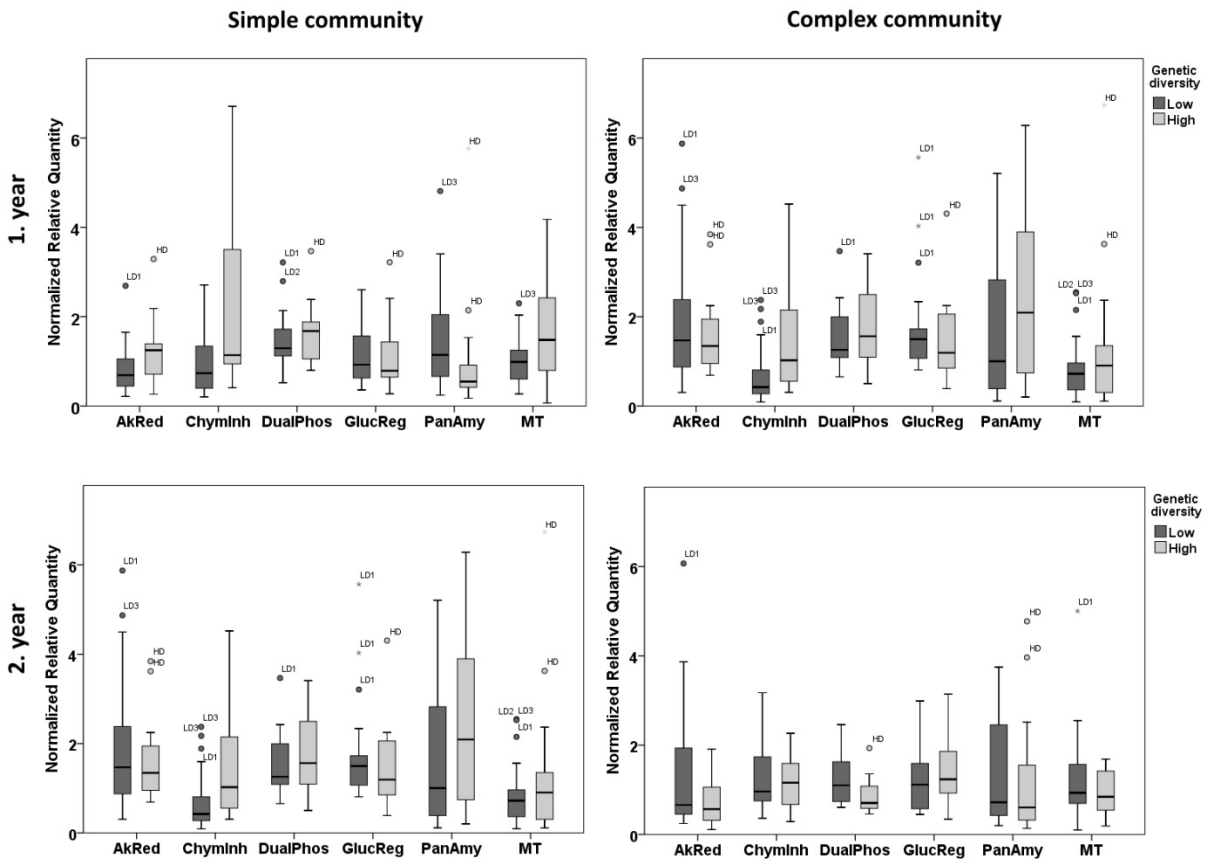

Figure 2. Gene expression (Normalized Relative Quantity) in the low diversity and high diversity treatments in the experiments with a simple or complex community of other decomposer animals.

Supplement to the manuscript: Impact of genetic diversity of an earthworm on decomposition and ecosystem functioning by Mustonen, M., Haimi, J. Knott, K.E.

Supplementary Material 3. Primer sequences used in qPCR and reaction efficiencies.

Gene	Primer (5'-3')	Efficiency %
MT-2	F: ACACTCAGTGCTGTGGCAGCG	103,5
	R: GGCTGCGCACTTGCAGGC	
AkRed	F: GCTGATCGAGGCAGATCCTT	96,2
	R: CCAAGTCCACGAACCCTGAA	
ChymInh	F: TCAGCTACCCGGTTCTAGGT	94,8
	R: AAGAGGCCAAAGCACAGGTT	
DualPhos	F: CGTCCAGTCATCCGTCCAAA	100,2
	R: ATAGCTCCAATGGGTGACGG	
GlucReg	F: GACGAGCTCATCAGTTGCAG	94,6
	R: TCTTTCAGGCGGAGGTTGAT	
PanAmy	F: ATCCTGACCTTCTTCGAGGC	97,9
	R: TCCTCGGCCAATAGTAGCTG	
18S	F: ACCACATCCAAGGAAGGCAG	93,3
	R: CCCGAGATCCAACTACGAGC	
28S	F: TGGTGGAGGTCCGCAGCGAT	90,5
	R: CGTTTCGTCCCCAAGGCCTC	