Impact of crop genetic diversity on a litter consumer

Pierre Chassé^{a,b,1}, Céline Pelosi^c, Jean-Christophe Lata^b, Sébastien Barot^b

^aDépartement de biologie, Ecole Normale Supérieure de Lyon, 15 parvis René Descartes,
69342 Lyon, France

^bIEES-Paris (CNRS, UPMC, IRD, INRA, UPEC), UPMC 4 place Jussieu, 75252 Paris cedex
05

^cUMR ECOSYS, INRA, AgroParisTech, Université Paris-Saclay, 78026 Versailles, France

Abstract

Belowground communities, and especially earthworms, provide numerous services in agroecosystems. In the current context of crop genetic erosion, this study aims at assessing the effect of wheat cultivar diversity on earthworm growth and survival. Our laboratory experiment was divided into two periods: (1) the "feeding period" (4 months), simulating conditions in autumn with cool temperatures and high food availability, and (2) the "non-feeding period" (1 month) simulating conditions in winter with cold temperatures and low food availability because litter had already been consumed without replacement. The aerial biomass of mixtures of wheat cultivars was used as food for earthworms and their growth was measured twice during the feeding period and once during the non-feeding period. We found two major effects on earthworm growth. Increasing the cultivar number (i) increased juvenile earthworm growth and earthworm survival and (ii) decreased adult earthworm growth. A consistent positive effect of nitrogen concentration of the biomass was found on growth and this effect was independent of biodiversity effects. This study suggests that the loss of litter

*Corresponding author.

E-mail address: pierre.chasse@ens-lyon.fr (P. Chassé).

intraspecific genetic diversity has an impact on earthworm populations, probably through mechanisms linked to the variance in edibility or the balanced diet hypotheses. While they remain to be tested in the field, these results suggest that the current losses of crop genetic diversity could potentially impact processes at the community or ecosystem level through a decrease in food quality.

Keywords: balanced diet hypothesis; cultivar mixture; earthworms; genetic diversity; mixed diet hypothesis; sustainable agriculture

Introduction

It is now well documented that species richness, mostly through functional diversity, strongly influences ecosystem functioning (Isbell et al., 2011; Cardinale et al., 2012). While most studies focus on species richness, recent studies are showing that genetic diversity within species can also influence species interactions and ecosystem processes (Hughes et al., 2008; Cook-Patton et al., 2011; Prieto et al., 2015) through variations in ecologically important traits within the species (Mcgill et al., 2006). Due to the fact that phenotypic diversity can be as high within species as among species (Bangert et al., 2005), it would not be surprising if interspecific diversity and intraspecific diversity had the same type of ecological consequences. Some recent results are in fact pointing in that direction (Creissen et al., 2013; Schöb et al., 2015), even if underlying mechanisms have not yet been fully documented for intraspecific genetic diversity.

The effect of horizontal biodiversity, i.e. within a trophic group, on adjacent trophic levels is, however, less known and more complicated to study (Duffy et al., 2007). Prey diversity, has been shown to influence ecosystem functioning through various mechanisms reviewed by Duffy et al. (2007). According to the balanced diet hypothesis, a more diverse resource assemblage leads to the consumption of more diverse nutritional resources, which

improves the growth of the consumer (DeMott, 1998; Pfisterer et al., 2003). On the other hand, according to the variance in edibility hypothesis, a more diverse range of resources likely contains resources resistant to consumption or at least less valuable in terms of nutrient content, which could decrease consumer growth (Leibold, 1989). These two mechanisms are not incompatible and can operate simultaneously. The balanced diet hypothesis relies on the fact that animals have particular food requirements, called “intake target”, for different nutrients such as proteins or carbohydrates (Senior et al., 2015). It has been shown that animals more easily reach their intake target with a diet composed of more than one type of food, i.e. a mixed diet (DeMott, 1998; Simpson et al., 2004; Senior et al., 2015). However, these experiments only manipulated species richness and not genetic intraspecific diversity. Our goal is thus to test whether genetic diversity within a plant species can impact its consumer through mechanisms linked to the balanced diet and variance in edibility hypotheses.

The intensification of agriculture has reduced crop biodiversity at different scales during the 20th century (FAO, 2010). In particular, genetically homogeneous crop cultivars have been selected and usually a single cultivar is planted within each field. However, more and more results are showing that mixing crop cultivars could be beneficial through an increase in production and a decrease in its temporal variability (Kiær et al., 2009; Barot et al., 2017). Among the identified mechanisms, a decrease in the sensitivity of crops to pathogens and parasites (Zhu et al., 2000) or to climate change (Tilman & Downing, 1994) seems to be particularly important. However, mixing crop cultivars could also promote the activity of beneficial organisms such as earthworms through the improvement of belowground-aboveground interactions (Postma-Blaauw et al., 2010). The influence of within field plant diversity loss on earthworm abundance and diversity is not yet fully demonstrated (Spehn et al., 2000; Laossi et al., 2008). Anecic earthworms in agricultural fields mostly feed

on litter composed of crop residues that are relatively poor in terms of nutrient compare to forest litter for instance. Yet, it is likely that earthworms could not reach their intake target for an optimal growth through this single genetically poor food resource available in this environment. If the balanced diet hypothesis applies to earthworms, genetic crop diversity erosion could affect earthworm growth and survival leading to a decrease in their activities and in the ecosystem services they provide. This is an important issue because: (1) Decomposers and especially earthworms provide many services to agriculture (Bertrand et al., 2015). Earthworms are known to contribute to physical, chemical and biological soil properties. Anecic earthworms accelerate the decomposition of plant litter and enhance water infiltration. (2) Current intensive agriculture has often deleterious effects on soil macrofauna (Birkhofer et al., 2008; Postma-Blaauw et al., 2010) and decrease earthworm abundance and diversity (Postma-Blaauw et al., 2010) through widespread practices such as tillage (Pelosi et al., 2009) and pesticides (Pelosi et al., 2014).

It is now well recognized that our current agricultural model based on the use of huge amounts of inputs and a few selected cultivars is not sustainable (Tilman et al., 2002; Foley et al., 2011). In order to maintain yields while developing a more sustainable agriculture, it is necessary to develop new alternative agricultural practices. The results of agroecological or ecological engineering experiments suggest that increasing biodiversity in agrosystems to mimic natural ecosystems could be a way to benefit from the services provided by biodiversity and consequently lower the use of chemical inputs (Malézieux, 2011). In this line of thought, this study aims at investigating the influence of mixtures of wheat cultivars on the growth and the survival of an anecic earthworm, *Lumbricus terrestris*. This species was selected for its crucial role in the decomposer communities of agricultural and forest ecosystems in temperate zones. Because food diversity could affect juvenile and adult earthworms differently, the aerial biomass of a field experiment was harvested and used as

food for earthworms at both the juvenile and adult life stage in a laboratory experiment. We assessed the effect of intraspecific genetic diversity of wheat cultivars and the effect of their functional diversity. We tested the general hypothesis that a high level of intraspecific diversity and functional diversity leads to high litter quality, providing more diverse nutrients to earthworms and consequently leading to higher growth and survival rates. This would support the balance diet hypothesis for earthworms. On the contrary, a negative effect of diversity would support the variance in edibility hypothesis.

Materials and methods

Wheat cultivars

The present experiment is based on a field experiment in small plots that is fully described elsewhere (Dubs et al., 2018). Wheat, *Triticum aestivum*, was used in the experiment. Twenty-four functional traits (See Appendix A: Table 1) were measured for 58 different cultivars. These cultivars were classified using their functional traits in a hierarchical cluster analysis, using Ward method into 4 "functional groups" (See Appendix A: Table 2). Four cultivars were selected from each of the 4 functional groups (16 cultivars in total). These 16 cultivars were chosen according to seed availability and in order to maximize the overall functional diversity, making sure that the clustering in four groups remained robust when the clustering process was applied to the 16 cultivars (Dubs et al., 2018). Among these cultivars, 9 were modern winter cultivars, selected to have high yields, 5 cultivars were old landraces, locally selected by farmers, and 2 cultivars were selected within an INRA MAGIC population, i.e. a highly recombinant and multi-parental population (60 parents: see Thepot et al. 2015 for more details). From these 16 different cultivars, 72 different combinations of wheat cultivars were created. Among these mixtures, 24, 28 and 20 mixtures respectively combined 2, 4 and 8 cultivars. Cultivars were selected in the mixtures in order to create, for

each level of cultivar diversity, homogeneous mixtures with low functional diversity (28 and 30 mixtures combined cultivars from only one or two functional groups respectively) and heterogeneous mixtures with high functional diversity (16 and 14 mixtures combined cultivars from three and four functional groups respectively) (See Appendix A: Table 3). The 16 plots with a single cultivar and the 72 plots with mixtures of cultivars were sown in Versailles (48°48'26"N 2°05'13"E) on November 2014. Earthworms were fed with 88 different litters coming from the aerial biomass of the 88 wheat mixtures harvested in May 2015. The aboveground biomass was dried (30 °C for 3 days) and cut (3 min in a blender) into small pieces (from 0.1 cm to 1 cm long).

The nitrogen concentration of each litter was measured on a sub-sample using an elemental analyzer (Flash HT, Thermo Scientific). The calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc concentrations were measured by dry mineralization and spectrometry (radial ICP-AES, Varian Vista RL).

Earthworm growth experiment

The soil used in the experiment was collected from the 0-30 cm soil layer of a wheat cultivated field. For technical reasons this was another field than the one used to grow the mixtures of cultivars. It was a sandy-loam soil made of 53% sand, 34% silt, 11% clay, and with 2.8% of organic matter. The soil was air-dried and manually homogenized.

L. terrestris were obtained from a commercial supplier (<http://appats-michel.fr/>). They were patted dry, weighed and classified into juveniles or adults based on the presence of a clitellum. 88 juveniles and 224 adults (average fresh weight $2.34\text{g} \pm 0.14\text{ g}$ for juveniles and $4.25\text{ g} \pm 0.11\text{ g}$ for adults) were used in the experiment. Each container (10 cm inner diameter, 15 cm height) was filled with 775 g of soil. The soil moisture was adjusted to 80% of the soil water holding capacity during the whole experiment (by weighing the pots every week). A single earthworm was placed in each container. The containers were covered with perforated

lids to prevent earthworm escape. Each earthworm was fed with the litter of a single experimental plot, i.e. a single mixture of wheat cultivar. The 16 treatments with litters obtained from one cultivar were replicated 6 times, one time with a juvenile and five times with an adult. The 72 treatments with litters combining more than one cultivar i.e. mixtures of cultivars, were replicated 3 times, one time with a juvenile and two times with an adult.

The experiment was divided into two periods to simulate local field conditions in cereal cultivation. The first one, simulating the autumn period, started in October 2015 and ended in February 2016. During this period called “feeding period”, all containers were kept at 15 °C and fed with 10 g of wheat litter by month (20 g of litter was added twice at the surface of the soil in October and December 2015). Earthworms were removed for weighing in February 2016 after little more than 3 months of experiment. Each earthworm was patted dry, weighed without voiding gut content (to avoid stress) and returned in its container. The second period, simulating winter and called “non-feeding period”, occurred between February and March 2016. Earthworms were kept at 8 °C without any new food supply. They were weighed in March 2016 using the method described above. Earthworm survival was assessed in December, in February and in March. During autumn, a significant quantity of litter is available at the soil surface (at least before tillage in agricultural systems) and earthworms are active. On the contrary, during winter, there is almost no litter available, temperature falls and earthworms are less active. The second period, between February and March, gave us information about a potential “carryover effect” (O’Connor et al., 2014), i.e. the capability of earthworms to survive and grow throughout winter depending on their previous feeding conditions. This experiment thus aims to study the influence of food genetic diversity on growth but also on the capability of earthworms to survive during winter.

168 *Calculation of earthworm growth rate*

169 We used the instantaneous growth rate (IGR, d⁻¹) (Daniel et al., 1996; Whalen & Parmelee,
170 1999), and calculated as,

171
$$\text{IGR} = \ln(W_t/W_i)/\Delta t,$$

172 where W_t (g) is the earthworm mass in February for the feeding period and in March for the
173 non-feeding period and W_i (g) the mass in October for the feeding period and the mass in
174 February for the non-feeding period. Δt was measured in days and represented the time
175 interval for the studied growth interval.

176 *Statistical analyses*

177 We used linear models to determine whether earthworm growth was influenced by the
178 mineral nutrient concentrations, the wheat cultivar number (WCN), wheat functional diversity
179 (as assessed by the number of cultivar functional groups in the mixtures of cultivars, F) and
180 the presence of each functional group (the effect of one particular functional group). The
181 number of cultivars and the number of functional groups within a mixture are not independent
182 because the number of functional groups cannot be higher than the number of cultivars and
183 conversely that mixtures of 8 cultivars have at least 2 functional groups. This is why we used
184 two different ANCOVA models to test the effect on earthworm growth of the
185 developmental stage (juvenile or adult, A), the effect of the wheat cultivar number or wheat
186 functional group, and the interaction between the two. We used an ANOVA model to test the
187 effect on earthworm growth of the developmental stage, the effect of the presence of each
188 functional group in the mixture and the interaction between the two. We considered the wheat
189 cultivar number and the wheat functional diversity as continuous variables to increase the
190 power of the tests and because we expected linear effects of diversity and tested their
191 interactions with the development stage. We first used linear mixed effect models, using the

composition of the cultivar mixture as a random effect, but as this random effect was never significant, we used standard models with only fixed effects. Homoscedasticity and normality of model residuals were tested to ensure the robustness of the results. Dead earthworms were removed from growth analyses. The influences of cultivar number, cultivar functional diversity and the presence of particular functional groups in the mixture on earthworm death were analyzed by logistic regressions (glm procedure using a logit link function with a binomial error) in three separate models to avoid any problem of dependence between tested factors. In these three models assessing the effect of wheat cultivar number, wheat functional diversity and the presence of particular functional groups in the mixture on earthworm growth, we also tested the effect of nutrient concentrations. Non-significant nutrients were removed manually from the final model. We used R software version 3.1.2 for all statistical analyses (R Development Core Team, 2008).

Results

General description of the results

By February, all juveniles were adults so that during the non-feeding period all earthworms were adults. However, the growth difference between young adults and adults was still significant during the October-February and the February-March periods so that we compared “juveniles” and “adults” in our statistical analyses. On average in all treatments, the biomass of juveniles increased by 309% ($\pm 8\%$) with a mean IGR of $1.1 \cdot 10^{-2} \text{ day}^{-1}$ ($\pm 0.6 \cdot 10^{-3} \text{ day}^{-1}$) in the feeding period. During the non-feeding period, juveniles lost 1% ($\pm 1\%$) of their weight. The biomass of adults increased by 168% ($\pm 4\%$) with a mean IGR of $5.1 \cdot 10^{-3} \text{ day}^{-1}$ ($\pm 0.2 \cdot 10^{-3} \text{ day}^{-1}$) in the feeding period. During the non-feeding period, adults lost 6% ($\pm 1\%$) of their weight. These results illustrate the effect of the developmental stage (Tables 1 and 2): juveniles thus grew faster than adults during the feeding period and lost less weight than

adults during the non-feeding period. However, in February, young adults had a lower weight than adults (result of t-test, $t_{148}=2.58$, $P=0.01$).

Influence of wheat cultivar number on earthworm growth

During the feeding period, no general effect of wheat cultivar number on earthworm growth was found but a significant interaction between development stage and wheat cultivar number occurred (Table 1). During the feeding period, juvenile growth significantly increased and adult growth significantly decreased with the number of cultivars (Fig. 1). During the non-feeding period, there was no significant effect of the cultivar number in feed mixtures that had been provided during the previous months on earthworm growth.

Influence of cultivar functional diversity on earthworm growth

During the feeding period, there was no general effect of cultivar functional diversity on earthworm growth (Table 2). During the non-feeding period, earthworms lost significantly less weight with an increasing functional diversity (Table 2, Fig. 2).

Influence of the presence of particular functional groups

Among the four functional groups, only one functional group had a significant influence on earthworm growth. Earthworms that ate wheat litter from functional group 3 gained less weight than the others (regression, $P<0.01$). When the mineral nutrient concentrations were added to the models, earthworm growth was no longer affected by cultivars from the functional group 3 but was positively affected by magnesium concentration (Table 3). When the negative effect of the functional group 3 was removed from the analyses, the growth of adult earthworms did not decrease anymore with increasing number of wheat cultivars or increasing wheat functional diversity (Table 3, Fig. 3). The growth of juvenile earthworms even increased significantly with an increasing number of wheat cultivars not belonging to the

functional group 3 during the feeding period (Table 3, Fig. 3). This suggested that the negative effect of cultivar number on adult earthworm growth was due to the presence of wheat from functional group 3 when the number of cultivars increases. During the non-feeding period, the effect of functional group 3 was the opposite: earthworms that had eaten litter from functional group 3 during the feeding period lost less weight than the others (Table 3).

Influence of nutrient concentrations on earthworm growth

Only nitrogen and magnesium had significant effects on earthworm growth (Tables 1 and 2). Nitrogen had a positive effect during the feeding periods and a negative effect during the non-feeding period. Magnesium had a positive effect during the feeding period. We found no interactions between the development stage and the different nutrient concentrations (statistical details not shown).

We also analyzed the influence of the wheat cultivar number, wheat functional diversity and the presence of particular functional groups on the two influential nutrients. There was no significant effect on nitrogen concentrations (result of linear models). There was a decrease in magnesium concentration with wheat cultivar (regression, $P=0.001$) number and wheat functional diversity (regression, $P=0.005$). Moreover, there was less magnesium in mixtures containing cultivars from the functional group 3 (ANOVA result, $P<0.001$, decrease in Mg content of 10% for mixtures with the functional group 3).

Influence of wheat cultivar number on earthworm mortality

Forty-one adults and 13 juveniles died during the October-December period. Between December and February, only 3% of the 258 remaining earthworms died: 8 adults and 1 juvenile. During the non-feeding period, only one juvenile died. Overall, over the feeding and non-feeding period, earthworm survival increased with the number of cultivars (logistic

regression, $P=0.0428$, Fig. 4). We did not find any significant effect of cultivar functional diversity or nutrient concentrations on earthworm mortality (statistical details not shown).

Discussion

Effect of cultivar mixtures during the Feeding period

During the feeding period, adult growth decreased with the number and the functional diversity of cultivars. This decrease could be explained by the variance in edibility hypothesis. This hypothesis is based on the fact that a more diverse range of resources likely contains resources resistant to consumption or at least less valuable in terms of nutrient content (Leibold, 1989). In our experiment, increasing the number of cultivars in mixtures increased the likelihood to have a cultivar from functional group 3, which has a negative effect on adult earthworm growth. To support the hypothesis that cultivars from functional group 3 triggered the negative effect of the increasing number of cultivars on adult earthworm growth, we analyzed the effect of an increase in the number of cultivars not belonging to the functional group 3. Since no relationship between adult growth and the number of cultivars not belonging to the functional group 3 has been found, this suggests that the decrease in adult earthworm growth can only be explained by the presence of cultivars from the functional group 3. Our results on nutrient concentration suggest that the negative effect of the functional group 3 is due to the lower magnesium concentration of the cultivars belonging to this functional group. We indeed found that the magnesium content of the wheat biomass had a positive effect on earthworm growth during the feeding period.

During the feeding period, juveniles behaved differently from adults since their growth increased with the number of cultivars. This result could be explained by the balanced diet hypothesis. This hypothesis is based on the fact that a more diverse resource assemblage leads to the consumption of more diverse nutritional resources, which improves the growth of the

consumer (DeMott, 1998; Pfisterer et al., 2003). Increasing the number of cultivars would lead to a better diet because cultivar diversity (apart from functional group 3) provides a more diverse range of nutrients for juvenile earthworms. To our knowledge, this is the first time intraspecific genetic diversity is shown to lead to the balanced diet or the variance in edibility hypothesis. Most of the experiments on the links between diet and animal growth and performance addressed the influence of interspecific resource diversity (DeMott, 1998; Pfisterer et al., 2003; Lefcheck et al., 2013). Most of them found positive effects of mixtures on the performance of consumers. DeMott (1998) suggested for instance that a diet composed of different prey species can be beneficial for its consumer. In this case, the different kinds of nutrients brought by each of the two prey species were complementary and increased consumer growth. A few studies observed negative effects of mixed diet on periphyton and amphibian biomass (Stoler & Relyea, 2011). Swan and Palmer (2006) documented an overall negative effect of interspecific litter diversity on detritivore growth. These opposite results may be related to the breadth of the diet of the consumer. Because some species have high requirements for particular nutrients, specialist consumers can be negatively impacted by a mixed diet because of the lower concentration of the food they particularly need. Generalist consumers likely have the opposite response. Earthworms are detritivores and *L. terrestris* feed on a wide range of organic materials and are considered generalist feeders (Curry & Schmidt, 2007). Our results support the idea that a more diverse intraspecific mix diet could provide a more diverse range of nutrients leading to a positive influence on generalist feeders. Overall, we hypothesize that depending on the consumer needs and the composition of its diet, either the balanced diet or the variance in edibility mechanisms, which have opposite consequences, are more influential.

In our experiment, it is likely that the variance in edibility hypothesis occurred in both adults and juveniles and the balanced diet hypothesis for juveniles only. There is already

evidence showing that food requirements are changing during an individual's life (Raubenheimer et al., 2007; Simpson & Raubenheimer, 2012). For instance, Dussutour and Simpson (2009) showed that ants, depending on their developmental stage, need a protein-rich diet or a carbohydrate-rich diet in order to maximize their performance (survival and production of larvae). The major difference between juvenile and adult earthworms is the presence of the reproductive organs. It has been shown that juveniles use food resources for the formation of sexual organs rather than their growth (Daniel et al., 1996). We could assume that juvenile earthworms that ate a more diverse range of nutrients (e.g. amino acids) were able to save energy – that they would otherwise have had to use to metabolize them – and allocated it both to the formation of their sexual organs and their growth. The nutrient intake target would be lower for adults than juveniles and could be reached with only one cultivar in adults, which would explain why there was no balanced diet effect in adults. However, the balanced diet effect on juvenile earthworm growth cannot be explained by the mineral nutrients we measured. The only effects of nutrients on earthworm growth during the feeding period were the positive effect of nitrogen and magnesium concentrations (see Table 1 and Table 2) but there was no relationship between nitrogen and wheat cultivar number and a negative relationship between magnesium and wheat cultivar number. In this case, no nutrient can explain the positive effect of cultivar numbers on earthworm growth. This suggests that another undocumented feature of wheat biomass was involved. We tried to assess the effect of other wheat functional traits measured on the 16 wheat cultivars but results remained difficult to interpret (See Appendix B). The positive effect of nitrogen concentration on earthworm growth during the feeding period is consistent with previous experiments on earthworms (Schonholzer et al. 1998; Eisenhauer et al. 2010).

We did not find any effect of an increase of functional diversity on earthworm growth. This result suggests that our approximation of functional diversity, i.e. the number of

functional groups in the litter, does not reflect a diversity affecting earthworm growth. Cultivars were classified using plant functioning and growth strategy traits (Wright et al., 2004). They might not be fully relevant to predict interactions with other trophic groups. However, it cannot be excluded that the positive effect of the number of cultivars is caused by a specific group of cultivars that has not been identified but whose likelihood of presence would increase with the number of cultivars. The use of traits more closely linked to litter quality and its nutritive value could help us to interpret our results and support either the variance in edibility or the balanced diet effect. In any case, whatever the underlying mechanism, we have demonstrated a trophic impact of intraspecific genetic diversity.

Effects of functional diversity during the non-feeding period

For technical reasons, the non-feeding period simulating winter only lasted one month but we can assume that the effect would have been amplified if the period had lasted longer. During the non-feeding period, earthworm growth increased with litter functional diversity. This result is consistent with the balanced diet hypothesis predicting that a mixed diet is beneficial. Moreover, we also found a positive effect of cultivars from functional group 3. Earthworms that ate cultivars from functional group 3 or litter composed of cultivars with a more diverse functional diversity during the feeding period lost less weight during the non-feeding period. Shipitalo et al. (1988) showed that *L. terrestris* ingested more slowly low-quality food than high-quality food. One explanation would then be that cultivars from functional group 3, providing a low-quality food (low magnesium concentration), were ingested more slowly than cultivar mixtures not containing wheat from this function group. Consequently, food containing cultivars from functional group 3 remained more available for earthworms during the non-feeding period, allowing them to lose less weight. This would also explain the negative influence of nitrogen concentration on earthworm growth during the non-feeding period. The low nitrogen concentration of particular cultivars would have decreased their

consumption rate during the feeding period and these low-quality cultivars (low nitrogen concentration) could still have been available during the non-feeding period for earthworms, allowing them to lose less weight.

Influence of the cultivar number on earthworm mortality

The mortality rate after December was similar or lower than in other earthworm laboratory studies despite the rather small size of the vials used in the present study (Daniel et al., 1996; Milcu et al., 2006). The higher mortality between October and December is probably due to the fact that earthworms came from a commercial supplier and had to adjust to the laboratory conditions. The positive effect of the number of cultivars on earthworm survival during the whole experiment was mainly due to the high mortality rate between October and December and suggests that the adaptation to the laboratory environment was facilitated by a more diverse food. This result on mortality is consistent with our results on earthworm growth supporting the balanced diet hypothesis. However, it remains difficult to determine whether this effect is driven by the balanced diet hypothesis, i.e. the positive influence of the interactions between cultivars, or the variance in edibility hypothesis, i.e. the positive influence of a particular cultivar or functional group that we did not identify. Such effects of interspecific food diversity on mortality have already been documented (Lefcheck et al., 2013). It has been shown for two bug species that larval survival was higher when they fed on several plants (Di Giulio & Edwards, 2003). Insect species likely depend on a specific nutrient balance, including amino acids, water content, minerals and trace elements that cannot be provided by a single-species diet but by the complementarity effect of a mixed diet. In our case, none of the studied mineral nutrients had an effect on earthworm mortality. However, other unmeasured characteristics of the biomass could explain the positive effect of the number of cultivars on earthworm survival.

Consequences for agricultural systems and ecosystems

The effect of crop genetic erosion on belowground communities has so far hardly been investigated. For instance, there is evidence suggesting that reducing plant species richness reduces earthworm species abundance in particular cases (Spehn et al., 2000). The underlying mechanism would be the reduction of primary production, which decreases the resources available to earthworms. Our results suggest that crop genetic erosion, besides its impact on crop production (Kiær et al., 2009; Borg et al., 2018), decreases earthworm growth and increases their mortality through the quality of the provided food. The decrease in earthworm abundance in cropping systems could thus be amplified by the intra-field reduction in crop genetic diversity. Since, the importance of services provided by earthworms to agriculture has been widely demonstrated (Bertrand et al., 2015), mixing cultivars could be a way to support earthworm populations and increase the services they provide. However, because the effects of cultivar mixtures on earthworm growth and mortality that we have documented in controlled conditions are rather small, field trials and modeling would be required to better assess the real and long term influence of these effects on earthworm populations in cropping systems.

However, to increase earthworm abundances using mixtures of cultivars it would first be necessary to determine the cultivar functional traits affecting earthworms in order to choose the right cultivars to be mixed. Indeed, as we saw with cultivars from functional group 3, mixing cultivars could have deleterious effects on earthworm growth. Moreover, the impact of crop genetic diversity we have detected on earthworms could also impact the whole soil biodiversity because a high proportion of soil organisms are decomposers that feed on litter. Hence, mixing cultivars could also affect the whole micro food-webs through the quality of the litter provided by mixtures. However, before using our results to improve agricultural practices, our results should be tested in field experiments and their robustness should be

assessed for other earthworm and soil fauna species and other crop types. Moreover, our results also depend on potential interactions between specific wheat cultivars and earthworm genotypes and we did not control for these genotypes. Earthworm genetic diversity is likely higher in agricultural fields and could influence the impact of the genetic diversity of their food.

Acknowledgments

The wheat biomass was produced by the experimental unit of INRA Versailles directed by C. Montagnier in an experiment coordinated by S. Saint-Jean, D. Tropée and P. Lusley and involving all the WHEATAMIX Consortium (www.inra.fr/Wheatamix). We thank M. Mendez from the Alysés analytical platform who has done the N analyses. We thank the INRA USRAVE laboratory for the other elemental analyses. This work was supported by the WHEATAMIX project, financed by the French National Research Agency (ANR-13-AGRO-0008).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XXXXX.

References

- Bangert, R.K., Turek, R.J., Martinsen, G.D., Wimp, G.M., Bailey, J.K., and Whitham, T.G., 2005. Benefits of Conservation of Plant Genetic Diversity to Arthropod Diversity. *Conserv. Biol.* 19, 379–390.
- Barot, S., V. Allard, A. Cantarel, J. Enjalbert, A. Gauffreteau, I. Goldringer, J.-C. Lata, X. Le Roux, A. Niboyet, and E. Porcher, 2017. Designing mixtures of varieties for multifunctional agriculture with the help of ecology. A review. *Agronomy for Sustainable Development* 37:13.

435 Bertrand, M., Barot, S., Blouin, M., Whalen, J., de Oliveira, T., and Roger-Estrade, J., 2015.
 436 Earthworm services for cropping systems. A review. *Agron. Sustain. Dev.* 35, 553–
 437 567.

438 Birkhofer, K., Bezemer, T.M., Bloem, J., Bonkowski, M., Christensen, S., Dubois, D.,
 439 Ekelund, F., Fließbach, A., Gunst, L., Hedlund, K., et al., 2008. Long-term organic
 440 farming fosters below and aboveground biota: Implications for soil quality, biological
 441 control and productivity. *Soil Biol. Biochem.* 40, 2297–2308.

442 Borg, J., L. Kiær, C. Lecarpentier, I. Goldringer, A. Gauffreteau, S. Saint-Jean, S. Barot, and
 443 J. Enjalbert. 2018. How to unfold the potential of cultivar mixtures in bread wheat: A
 444 meta-analysis perspective on diversification strategies and identification of lacks of
 445 knowledge. *Field Crop Research* 221:298-313.

446 Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., et al., 2012.
 447 Biodiversity loss and its impact on humanity. *Nature.* 486 (7401), 59-67.

448 Cook-Patton, S.C., McArt, S.H., Parachnowitsch, A.L., Thaler, J.S., and Agrawal, A.A., 2011.
 449 A direct comparison of the consequences of plant genotypic and species diversity on
 450 communities and ecosystem function. *Ecology* 92, 915–923.

451 Creissen, H.E., Jorgensen, T.H., and Brown, J.K.M., 2013. Stabilization of yield in plant
 452 genotype mixtures through compensation rather than complementation. *Ann. Bot.* 112,
 453 1439–1447.

454 Curry, J.P., and Schmidt, O., 2007. The feeding ecology of earthworms – A review.
 455 *Pedobiologia* 50, 463–477.

456 Daniel, O., Kohli, L., and Bieri, M., 1996. Weight gain and weight loss of the earthworm
 457 *Lumbricus terrestris* L. at different temperatures and body weights. *Soil Biol.*
 458 *Biochem.* 28, 1235–1240.

459 DeMott, W.R., 1998. Utilization of a cyanobacterium and a phosphorus-deficient green alga
 460 as complementary resources by daphnids. *Ecology* 79, 2463–2481.

461 Di Giulio, M., and Edwards, P.J., 2003. The influence of host plant diversity and food quality
 462 on larval survival of plant feeding heteropteran bugs. *Ecol. Entomol.* 28, 51–57.

463 Dubs, F., Le Roux, X., Allard, V., Andrieu, B., Barot, S., Cantarel, A., Vallavielle-Pope, C.
 464 de, Gauffreteau, A., Goldringer, I., Montagnier, C., et al. (2018). An experimental
 465 design to test the effect of wheat variety mixtures on biodiversity and ecosystem
 466 services. (<https://hal.archives-ouvertes.fr/hal-01843564>)

467 Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E., and Loreau, M., 2007.
 468 The functional role of biodiversity in ecosystems: incorporating trophic complexity.
 469 *Ecol. Lett.* 10, 522–538.

470 Dussutour, A., and Simpson, S.J., 2009. Communal Nutrition in Ants. *Curr. Biol.* 19, 740–
 471 744.

472 Eisenhauer, N., Butenschoen, O., Radsick, S. & Scheu, S., 2010. Earthworms as seedling
473 predators: Importance of seeds and seedlings for earthworm nutrition. *Soil Biology*
474 *and Biochemistry* 42, 1245–1252.

475 FAO, 2010. The second report on the state of the world's plant genetic resources for food and
476 agriculture. FAO, Rome.

477 Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS et al., 2011. Solutions for a
478 cultivated planet. *Nature* 478 (7369):337-342.

479 Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., et al., 2011.
480 High plant diversity is needed to maintain ecosystem services. *Nature*. 477 (7363),
481 199-202.

482 Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N., and Vellend, M., 2008.
483 Ecological consequences of genetic diversity: Ecological effects of genetic diversity.
484 *Ecol. Lett.* 11, 609–623.

485 Kiær, L.P., Skovgaard, I.M., and Østergård, H., 2009. Grain yield increase in cereal variety
486 mixtures: A meta-analysis of field trials. *Field Crops Res.* 114, 361–373.

487 Laossi, K.-R., Barot, S., Carvalho, D., Desjardins, T., Lavelle, P., Martins, M., Mitja, D.,
488 Carolina Rendeiro, A., Rousseau, G., Sarrazin, M., et al., 2008. Effects of plant
489 diversity on plant biomass production and soil macrofauna in Amazonian pastures.
490 *Pedobiologia* 51, 397–407.

491 Lefcheck, J.S., Whalen, M.A., Davenport, T.M., Stone, J.P., and Duffy, J.E., 2013.
492 Physiological effects of diet mixing on consumer fitness: a meta-analysis. *Ecology*,
493 94: 565–572.

494 Leibold, M.A., 1989. Resource Edibility and the Effects of Predators and Productivity on the
495 Outcome of Trophic Interactions. *Am. Nat.* 134, 922–949.

496 Malézieux, E., 2011. Designing cropping systems from nature. *Agronomy for Sustainable*
497 *Development* 32:15-29.

498 McGill, B., Enquist, B., Weiher, E., and Westoby, M., 2006. Rebuilding community ecology
499 from functional traits. *Trends Ecol. Evol.* 21, 178–185.

500 Milcu, A., Patsch, S., Langel, R., and Scheu, S., 2006. The response of decomposers
501 (earthworms, springtails and microorganisms) to variations in species and functional
502 group diversity of plants. *Oikos* 112, 513–524.

503 O'Connor, C.M., Norris, D.R., Crossin, G.T., and Cooke, S.J. (2014). Biological carryover
504 effects: linking common concepts and mechanisms in ecology and evolution.
505 *Ecosphere* 5, art28.

506 Pelosi, C., Bertrand, M., and Roger-Estrade, J., 2009. Earthworm community in conventional,
507 organic and direct seeding with living mulch cropping systems. *Agron. Sustain. Dev.*
508 29, 287–295.

509 Pelosi, C., Barot, S., Capowiez, Y., Hedde, M., and Vandebulcke, F., 2014. Pesticides and
510 earthworms. *Agron. Sustain. Dev.* 34, 199–228.

511 Pfisterer, A.B., Diemer, M., and Schmid, B., 2003. Dietary shift and lowered biomass gain of
512 a generalist herbivore in species-poor experimental plant communities. *Oecologia* 135,
513 234–241.

514 Postma-Blaauw, M.B., de Goede, R.G.M., Bloem, J., Faber, J.H., and Brussaard, L., 2010.
515 Soil biota community structure and abundance under agricultural intensification and
516 extensification. *Ecology* 91, 460–473.

517 Prieto, I., Violle, C., Barre, P., Durand, J.-L., Ghesquiere, M., and Litrico, I., 2015.
518 Complementary effects of species and genetic diversity on productivity and stability of
519 sown grasslands. *Nat. Plants* 1, 15033.

520 R Development Core Team, 2008. R: A language and environment for statistical computing.
521 R Foundation for Statistical Computing, Vienna, Austria. (<http://www.R-project.org>).

522 Raubenheimer, D., Mayntz, D., Simpson, S.J., and Tøft, S., 2007. Nutrient-specific
523 compensation following diapause in a predator: implications for intraguild predation.
524 *Ecology* 88, 2598–2608.

525 Schöb, C., Kerle, S., Karley, A.J., Morcillo, L., Pakeman, R.J., Newton, A.C., and Brooker,
526 R.W., 2015. Intraspecific genetic diversity and composition modify species-level
527 diversity-productivity relationships. *New Phytol.* 205, 720–730.

528 Schonholzer, F., Kohli, L., Hahn, D., Daniel, O., Goez, C. and Zeter, J. 1998. Effects of
529 decomposition of leaves on bacterial biomass and on palatability to *Lumbricus*
530 *terrestris* L. *Soil Biol. Biochem.* 30: 1 805–1 813.

531 Senior, A.M., Nakagawa, S., Lihoreau, M., Simpson, S.J., and Raubenheimer, D., 2015. An
532 Overlooked Consequence of Dietary Mixing: A Varied Diet Reduces Interindividual
533 Variance in Fitness. *Am. Nat.* 186, 649–659.

534 Shipitalo, M., Protz, R. & Tomlin, A. (1988). Effect of diet on the feeding and casting activity
535 of *Lumbricus terrestris* and *L. rubellus* in laboratory culture. *Soil Biology and*
536 *Biochemistry*, 20(2), 233–237.

537 Simpson, S.J. & Raubenheimer, D., 2012. *The Nature of Nutrition: A Unifying Framework*
538 *from Animal Adaptation to Human Obesity*. Princeton University Press.

539 Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T., and Raubenheimer, D., 2004. Optimal
540 foraging when regulating intake of multiple nutrients. *Anim. Behav.* 68, 1299–1311.

541 Spehn, E.M., Joshi, J., Schmid, B., Alpehi, J., and Körner, C., 2000. Plant diversity effects on
542 soil heterotrophic activity in experimental grassland ecosystems. *Plant Soil* 224, 217–
543 230.

544 Stoler, A.B., and Relyea, R.A., 2011. Living in the litter: the influence of tree leaf litter on
545 wetland communities. *Oikos* 120, 862–872.

- 546 Swan, C.M., and Palmer, M.A. (2006). Preferential Feeding by an Aquatic Consumer
547 Mediates Non-Additive Decomposition of Speciose Leaf Litter. *Oecologia* 149, 107–
548 114.
- 549 Thepot, S., Restoux, G., Goldringer, I., Hospital, F., Gouache, D., Mackay, I., and Enjalbert,
550 J., 2015. Efficiently Tracking Selection in a Multiparental Population: The Case of
551 Earliness in Wheat. *Genetics* 199, 609–623.
- 552 Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S, 2002. Agricultural sustainability
553 and intensive production practices. *Nature* 418:671-677.
554
- 555 Tilman, D., and Downing, J.A., 1994. Biodiversity and stability in grasslands. *Nature* 367,
556 363–365.
- 557 Whalen, J.K., and Parmelee, R.W., 1999. Growth of *Aporrectodea tuberculata* (Eisen) and
558 *Lumbricus terrestris* L. under laboratory and field conditions. *Pedobiologia* 43, 1–10.
- 559 Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-
560 Bares, J., Chapin, T., Cornelissen, J.H., Diemer, M., et al., 2004. The worldwide leaf
561 economics spectrum. *Nature* 428, 821–827.

Table 1. Results of two linear models analyzing earthworm growth (IGR) as a function of the developmental stage (A), mineral nutrient concentrations (N: nitrogen, Mg: magnesium), wheat cultivar number (WCN) and their interactions during the two periods: FP for the feeding period and NFP for non-feeding period. The non-significant variables and interactions are removed from the models (e.g. other mineral nutrient concentrations). The negative effect “(-)” or the positive effect “(+)” of the variables are indicated in the P column for each period. In case of significant interaction between the maturity stage and the cultivar number the effect (+ or -) of cultivar number is indicated separately for adults (Ad) and juveniles (Juv).

	FP			NFP		
	DF	F	P	DF	F	P
A	1	517.00	<0.001	1	17.0	<0.001
N	1	4.28	0.040 (+)	1	4.2	0.040 (-)
Mg	1	5.55	0.020 (+)			NS
WCN	1	1.59	NS			NS
A×WCN	1	13.20	<0.001			NS
<i>Ad: - ; Juv: +</i>						
Total	248			247		
		R² = 0.68			R² = 0.07	

Table 2. Results of a linear model analyzing earthworm growth (IGR) as a function of the developmental stage (A), mineral nutrient concentrations (N: nitrogen, Mg: magnesium), wheat functional diversity (F, number of functional groups in the mixtures of cultivars) and their interactions during the two periods: FP for the feeding period and NFP for non-feeding period. The non-significant variables and interactions are removed from the models (e.g. other mineral nutrient concentrations). The negative effect “(-)” or the positive effect “(+)” of the variables are indicated in the P column for each period. In case of significant interaction between the maturity stage and the cultivar number the effect (+ or -) of cultivar number is indicated separately for adults (Ad) and juveniles (Juv).

	FP			NFP		
	DF	F	P	DF	F	P
A	1	494.00	<0.001	1	17.0	<0.001
N	1	4.09	0.044 (+)	1	4.3	0.040 (-)
Mg	1	5.30	0.022 (+)			NS
F	1	0.11	NS	1	7.0	0.008 (+)
A×F	1	3.15	<i>0.077</i>			NS
<i>Ad: - ; Juv: No effect</i>						
Total	248			247		
		R² = 0.67			R² = 0.09	

Table 3. Results of a linear model analyzing earthworm growth (IGR) as a function of the developmental stage (A), mineral nutrient concentrations (N: nitrogen, Fe: iron, Mg: magnesium), the presence of cultivars belonging to particular functional groups (functional groups from C1 to C4, C: functional group), the number of cultivars not belonging to the third functional group (N≠C3) and their interactions during the two periods: FP for the feeding period with the effect of magnesium and FP (Mg-) for the feeding period without the effect of magnesium in the model, and NFP for the non-feeding period. Non-significant variables (especially the presence of cultivars from functional group 1, 2, 4 and other mineral nutrient concentrations) have been removed manually for the final model. The negative effect “(-)” or the positive effect “(+)” of the variables are indicated in the P column for each period. In case of significant interaction between the maturity stage and the cultivar number the effect (+ or -) of cultivar number is indicated separately for adults (Juv) and juveniles (Ad).

	FP		FP (Mg-)		NFP	
	F	P	F	P	F	P
A	515.0	<0.001	511.0	<0.001	17.0	<0.001
N	4.3	0.040 (+)	4.2	0.040 (+)	4.3	0.040 (-)
Mg	5.5	0.020 (+)	-	-		NS
C3	2.3	NS	5.6	0.018 (-)	5.7	0.020 (+)
WCN≠C3	2.1	NS	1.3	NS		NS
A×WCN≠C3	10.0	0.002	9.9	0.002		NS
	Ad: No effect ; Juv: +		Ad: No effect ; Juv: +			
Total DF		248		248		247
R²		0.68		0.68		0.09

Fig. 1. Covariance analysis of earthworm growth as a function of the number of cultivars and earthworm stage, i.e. juvenile vs. adult during the feeding period. The trend lines are represented by the equations $y = 0.00027x + 0.01$ (juveniles, $P = 0.02$) and $y = -0.0001x + 0.00544$ (adults, $P = 0.03$). The confidence intervals (95%) of the regression fits are represented by the grey area.

Fig. 2. Effect of wheat functional diversity on earthworm growth during the non-feeding period. The trend line is represented by the equation $y = 0.00055x - 0.00273$ ($P = 0.001$). The confidence interval (95%) of the regression fit is represented by the grey area.

Fig. 3. Effect of the number of wheat cultivars not belonging to the functional group 3 on earthworm growth, depending on their age. The trend lines are represented by the equations $y = 0.00032x + 0.01$ (juveniles, $P = 0.02$) and $y = -0.00008x + 0.00531$ (adults, $P = 0.15$). The confidence intervals (95%) of the regression fits are represented by the grey area.

Fig. 4. Logistic regression between the mortality probability and the number of cultivars. Mortality probability is indicated in the graph for each number of cultivars. $P(D/N=1) = 0.247$, $P(D/N=2) = 0.225$, $P(D/N=4) = 0.186$, $P(D/N=8) = 0.124$. N, number of cultivars; $P(D/i)$, the probability of mortality if there are i cultivars. Probabilities are indicated by “o”. $P\text{-value} = 0.043$. Confidence interval (95%) of the regression fit is represented by the grey area.

626 Fig. 1

627

628

629

630

631 Fig. 2

632

633

634

Fig. 3

Fig. 4