Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2021.014968

ARTICLE

Effects of Soil Nutrient Heterogeneity and Earthworms on Aboveground Biomass of Experimental Plant Communities

Simei Yao[1,2], Yu Jin[2,3], Limin Zhang[2], Ningfei Lei[1,*], Wei Xue[2,*], Jinsong Chen[3], Qian Zhang[2] and Feihai Yu[1,2]

[1]College of the Environment, Chengdu University of Technology, Chengdu, 610059, China

[2]Institute of Wetland Ecology & Clone Ecology, Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou, 318000, China

[3]College of Life Science, Sichuan Normal University, Chengdu, 610101, China

[*]Corresponding Author: Ningfei Lei. Email: leiningfei@cdut.cn; Wei Xue. Email: x_wei1988@163.com

Received: 12 November 2020 Accepted: 12 January 2021

ABSTRACT

Soil nutrients are commonly heterogeneously distributed and earthworms are one of the most common soil organisms. While effects of both soil nutrient heterogeneity and earthworms have been well studied, their interactive effect on plant community productivity has rarely been tested. In a greenhouse experiment, we constructed experimental plant communities by sowing seed mixtures of four grasses, two legumes and two forbs in either a heterogeneous soil consisting of low and high nutrient soil patches or a homogeneous soil where the low and high nutrient soil patches were evenly mixed. The earthworm *Eisenia fetida* was either added to these soils or not. Aboveground biomass of the whole communities, grasses and legumes did not differ between the homogeneous and heterogeneous soils or between the soils with and without earthworms. However, soil nutrient heterogeneity reduced aboveground biomass of forbs, and such an effect did not interact with earthworms. In response to soil heterogeneity and earthworms, biomass ratio of the three functional groups showed similar patterns as that of their biomass. At the patch level, aboveground biomass of the whole community, grasses and legumes were greater in the high than in the low nutrient soil patches within the heterogeneous soil. A similar pattern was found for the forbs, but this was only true in the absence of earthworms. Our results suggest that soil nutrient heterogeneity and earthworms may not influence aboveground biomass of plant communities, despite the fact that they may modify the growth of certain plant functional groups within the community.

KEYWORDS

Environmental heterogeneity; foraging response; functional group; plant-animal interaction; yield

1 Introduction

Soil heterogeneity is one of the inherent characteristics of natural habitats [1–3]. Theoretical models have shown that soil heterogeneity can greatly influence plant performance [4,5], and this view was supported by many empirical studies [6–9]. Plants, especially those capable of rapid clonal growth, can benefit from the heterogeneous distribution of soil resources [10–13], as they can efficiently utilize these resources from microsites of high concentrations (i.e., high resource patches) [14,15]. In a heterogeneous soil, plant species differing in growth strategies may tend to occupy different soil patches, allowing more species to coexist and consequently a more complementary resource utilization [16,17]. Therefore, soil resource heterogeneity may enhance the productivity of plant communities [18–20].

As ecosystem engineers, earthworms have substantial contributions to the formation and modification of the soil where plants can grow [21]. They may accelerate the degradation of soil organic matter [22,23], change soil nutrient availability [22,24], and alter soil water holding capacity [25,26]. These earthworm-induced changes of the soil may thus indirectly influence plant performance [27]. Earthworms can also influence plant performance directly; for example, they may reduce plant growth through consumption of plant seedlings and burying of plant seeds [28–30]. However, they may promote plant growth through releasing of hormone-like chemicals that can increase the plant resistance ability to diseases [31,32].

The presence of earthworms may change the effects of soil nutrient heterogeneity on plant communities. The effectiveness of soil nutrient heterogeneity experienced by a plant community may be reduced as the earthworms may homogenize the heterogeneous soil by either ingesting or excreting it on high or low nutrient patches, respectively [33,34]. Earthworms may also increase the effectiveness of soil nutrient heterogeneity experienced by plant communities. This is because they can stabilize the organic matter in the soil and form soil aggregates through bioturbation [35–37]. Liu et al. [38] found that the presence of earthworms can reduce the positive effects of soil nutrient heterogeneity on the growth of grasses, but this effect varied between plant species. Therefore, we expect that earthworms and soil nutrient heterogeneity may also interact to influence the performance of plant communities consisting of many species of contrasting growth strategies.

To investigate how soil nutrient heterogeneity and earthworms may influence plant community performance, we created a plant community in a greenhouse experiment. This was made by sowing a seed mixture of four grasses, two legumes and two forbs in an either heterogeneous soil consisting of low and high nutrient soil patches or homogeneous soil where the low and high nutrient soil patches were evenly mixed. Each soil was added either with or without earthworms. Specifically, we tested the following hypotheses: (1) plant communities produce more biomass in heterogeneous than homogeneous soils; (2) plant communities produce more biomass in the soil with than without earthworms; (3) the presence of earthworms reduces the positive effects of soil nutrient heterogeneity on plant communities.

2 Materials and Methods

2.1 Experimental Mesocosms

Grassland species are generally classified into three functional groups (i.e., grasses, legumes and forbs) based on their traits associated with the response variables (e.g., biomass production, resource use strategy, N-fixation ability). The species of the three functional groups are widely distributed in grasslands [39]. We constructed experimental communities using eight plant species from the three functional groups [i.e., four grasses (*Bromus inermis* Leyss., *Lolium perenne* L., *Elymus dahuricus* Turcz. and *Festuca elata* Keng ex E. Alexeev), two legumes (*Trifolium repens* L. and *Medicago sativa* L.) and two forbs (*Plantago asiatica* L. and *Oxalis corniculata* L.)]. All these species are perennial and commonly found in grasslands [40]. Seeds of the eight species were purchased from the Agricultural Institute of Jiangsu Province, China, and stored at 4°C before use.

Eisenia fetida Savigny (Lumbricidae, epigeic redworm) is a common earthworm species worldwide [41,42]. It is generally 6 to 8 cm long and active mainly in the upper soil from the soil surface. This species is originally from Europe, but it was introduced into other continents. In China, *E. fetida*is widely distributed in commercial composting areas, semi-natural grasslands and farmlands [43]. The earthworms used in this experiment were purchased from the earthworm breeding base in Zhenjiang, Jiangsu Province, China.

2.2 Experimental Design

The experiment used a factorial design with two soil treatments (homogeneous and heterogeneous) crossed with two earthworm treatments (with and without). Each of the four combinations of treatments was replicated 10 times. The communities were each constructed in a container of 45 cm long × 45 cm wide × 35 cm deep. Each container was divided into 16 equal quadrants of 11 cm × 11 cm (Fig. 1). In the heterogeneous soil treatment, eight quadrants in the container were filled with a high nutrient soil and the other eight in that container with a low nutrient soil to a depth of 30 cm. In the homogeneous treatment, each quadrant was filled with an even mixture of the high and low nutrient soils to the same depth. The high nutrient soil was an 1:1 (v:v) mixture of local soil and peat with 4 g L^{-1} slow release fertilizer (14N: 14P: 14K; Osmocote, Scotts, USA), and the low-nutrient soil was an 1:1 (v:v) mixture of local soil and peat without slow release fertilizer. In this way, the homogeneous and heterogeneous soils had a distinct spatial configuration but an equivalent initial total amount of nutrients. Each homogeneous and heterogeneous treatment had 20 replicates (containers).

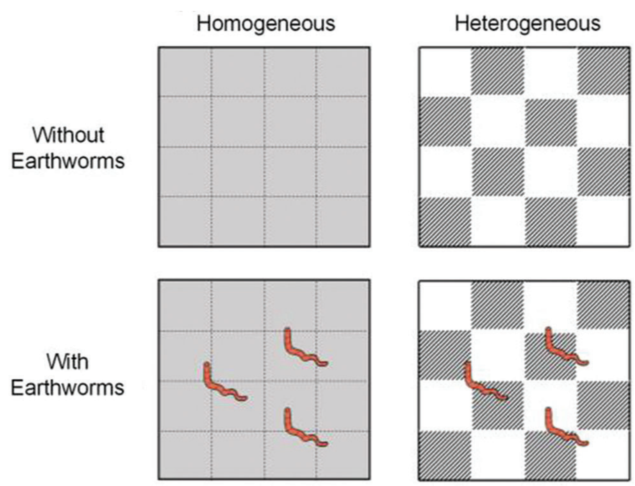

Figure 1: Schematic diagram of the experimental design. The experiment consisted of two soil treatments (homogeneous *vs.* heterogeneous) crossed with two earthworm treatments (with *vs.* without). Seeds of eight grassland species were sown in containers that were each divided into 16 patches of equal size. In the heterogeneous treatment, eight patches were filled with a high nutrient soil, and the other eight with a low nutrient soil. In the homogeneous treatment, each of the 16 patches in the container was filled with an 1:1 mixture of the high and low nutrient soils. For both homogeneous and heterogeneous treatments, half of the containers were each added with 30 earthworms (*Eisenia fetida*) while the other half received no earthworms

On 06 July 2018, we sowed two seeds of each of the eight species on the soil surface of each quadrant in each container and covered them with a layer of soil. We thus sowed a total of 256 seeds in each container. The initial composition of the community was visibly identical (personal observation), and approximately 50–60 seedlings were established in each pot. Three weeks later (on 28 July 2018), in each soil treatment, we randomly selected half (10) of the containers and added 30 individuals of *E. fetida* (earthworm) of similar size in each of them. This provided a density of about 150 earthworms m^{-2}, slightly higher than the range of common densities in arable soils, but lower than that in some coniferous forests [44]. The remaining ten containers in each soil treatment were not added with any earthworms.

The experiment ended on 28 August 2018. During the experiment, the mean temperature and relative humidity in the greenhouse were 28°C and 65%, respectively (iButtons; DS1923, Maxim Integrated

Products, Sunnyvale, CA, USA). Each pot was watered every two days. After the release of earthworms, each pot was watered twice a week and the soil moisture content was kept around 20%. Weeds from the soil seed bank were removed by hands during the experiment.

2.3 Harvest and Measurement

In the heterogeneous treatments, the aboveground part of the plants in the low and high nutrient soil patches (quadrants) in each container was harvested separately and sorted into functional groups (grasses, legumes and forbs). In the homogeneous treatments, the aboveground part of plants was harvested in a similar way (i.e., separately in the patches located in the high and low nutrient patches in the heterogeneous soil), and these patches were referred to as "imagined high nutrient patches" and "imagined low nutrient patches", respectively. This was done to generate a comparison between the rooting responses [i.e., the extent to which the plants could place their roots/shoots in the either high or imagined high nutrient soil patches] of the plant community in the heterogeneous and homogeneous soils. Plant parts were dried at 75°C for 48 h and weighed. Two replicates of the heterogeneous treatment with earthworms were destroyed due to carelessness, and were thus excluded from data analysis.

2.4 Statistical Analysis

Total aboveground biomass of a community in a container was the sum of biomass of the three functional groups in the container. We calculated functional group biomass ratio as aboveground biomass of a functional group (i.e., grasses, legumes or forbs) divided by total aboveground biomass of the community.

At the whole container level, we used two-way ANOVAs to test the effects of soil nutrient heterogeneity (homogeneous or heterogeneous) and earthworms (with or without) on total aboveground biomass of the whole community, and aboveground biomass and biomass ratio of each functional group. At the patch level, we used three-way ANOVAs with repeated measures to test the effects of soil heterogeneity, earthworms and patch quality (high or "imagined high nutrient quadrants" *vs.* low or "imagined low nutrient quadrants") on total aboveground biomass of the community, and aboveground biomass and biomass ratio of each functional group. In these models, patch quality was treated as a repeated measure because plant performance in the (imagined) low and (imagined) high nutrient soil patches within a container was not independent. Analyses were conducted using SPSS 22.0 (IBM Corp., Armonk, NY, USA). All data were checked for normality and homogeneity of variance.

3 Results

At the whole container level, total aboveground biomass of the whole community, or aboveground biomass of grasses or legumes was not significantly different between the homogeneous and heterogeneous soils (Figs. 2A–2C; Tab. 1). However, aboveground biomass of forbs was significantly lower in the heterogeneous than in the homogeneous soils (Fig. 2D; Tab. 1). Earthworms or their interaction with soil nutrient heterogeneity influenced neither aboveground biomass of the community nor that of each functional group (Fig. 2; Tab. 1). Biomass ratio of each of the three functional groups showed a similar pattern as that of its biomass (Fig. 3; Tab. 1).

At the patch level, total biomass and legume biomass were overall greater, and grass biomass tended to be greater in the high than in the low nutrient soil patches (Figs. 4A–4C; Tab. 2). Without earthworms, forbs tended to produce greater biomass in the high than in the low nutrient soil patches, but the reverse was true in the presence of earthworms (Fig. 4D; Tab. 2: a marginally significant effect of patch quality × earthworms). Patch quality or its interaction with soil nutrient heterogeneity and/or earthworms did not influence grass or legume biomass ratios (Figs. 5A and 5B; Tab. 2). Averaged across the two soil nutrient treatments, forb biomass ratio was greater in the high than in the low nutrient soil patches without earthworms, but tended to be smaller with earthworms, as indicated by the significant interaction effect of patch quality × earthworms (Fig. 5C; Tab. 2).

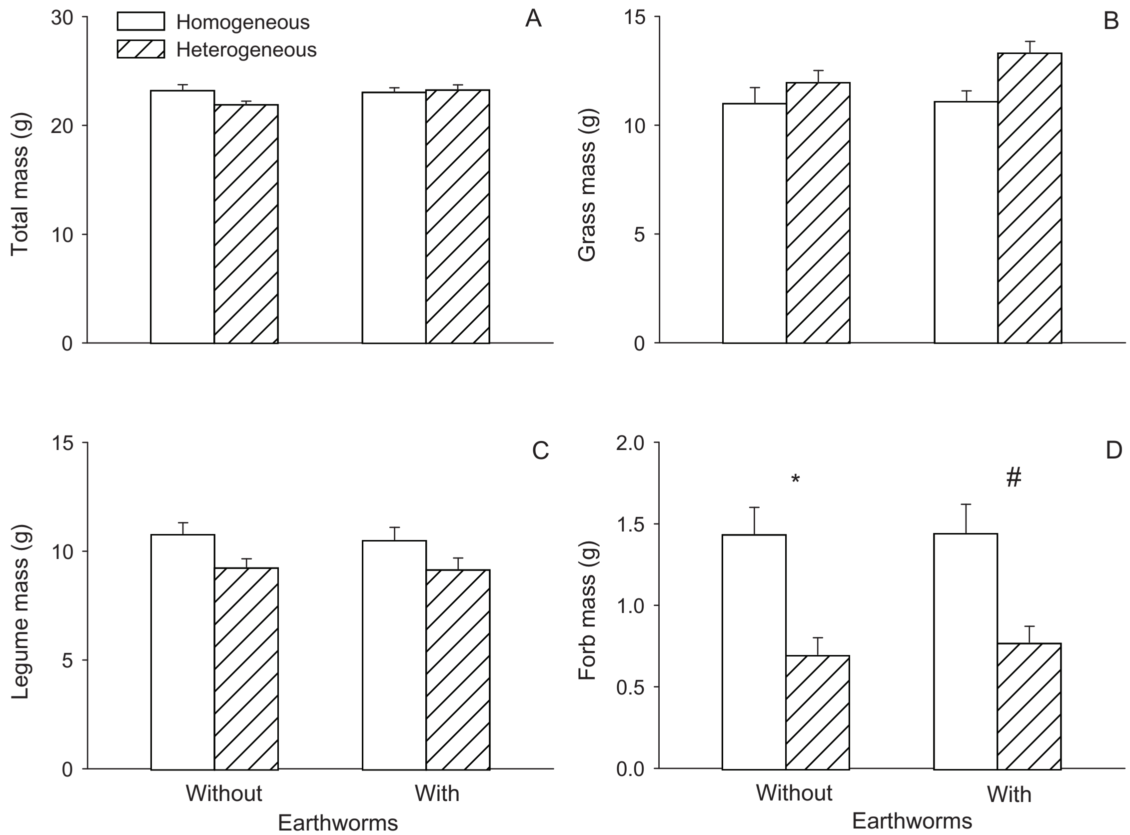

Figure 2: (A) Total aboveground biomass of the communities and (B–D) aboveground biomass of the three functional groups. Means + 1 SE are shown. Symbols (*$p < 0.05$ and $^{\#}p < 0.1$) indicate significant differences between the homogeneous and heterogeneous soils within each earthworm treatment

Table 1: ANOVA results for the effects of soil nutrient heterogeneity and earthworms on total aboveground biomass of the community, and aboveground biomass and aboveground biomass ratio of each functional group

Variable	Heterogeneity (H)		Earthworms (E)		H × E	
	$F_{1, 34}$	p	$F_{1, 34}$	p	$F_{1, 34}$	p
Total mass	0.41	0.525	0.49	0.489	0.82	0.372
Grass mass	1.87	0.180	0.39	0.537	0.30	0.590
Legume mass	2.10	0.157	0.03	0.857	0.01	0.925
Forb mass	**6.21**	**0.018**	0.02	0.886	0.02	0.905
Grass mass ratio	2.86	0.100	0.32	0.575	0.01	0.905
Legume mass ratio	1.46	0.236	0.40	0.530	0.02	0.894
Forb mass ratio	**7.04**	**0.012**	<0.01	0.986	<0.01	0.999

Note: Degree of freedom, F and p values are given.

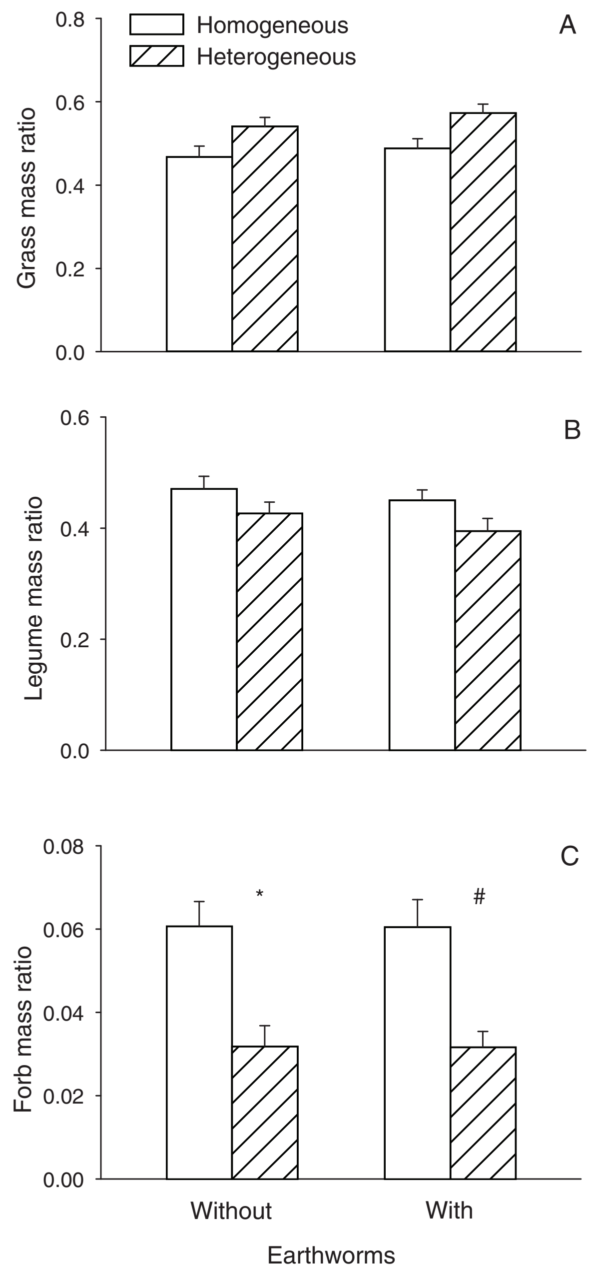

Figure 3: Aboveground biomass ratio of the (A) grasses, (B) legumes and (C) forbs. Means + 1 SE are shown. Symbols (*p < 0.05 and $^{\#}p$ < 0.1) indicate significant differences between the homogeneous and heterogeneous soils within each earthworm treatment

4 Discussion

The niche-based theory indicates that soil heterogeneity can promote plant species coexistence and thus enhance community productivity due to a more complementary utilization of the soil resources [45–47]. Our results did not support this view as we found that the plant community performed equally well in the homogeneous and heterogeneous soils. This indicated that soil nutrient heterogeneity did not necessarily support a greater growth of the plant community. The failure of soil nutrient heterogeneity in promoting plant community growth has generally been attributed to a smaller size of plant rooting systems than the size of soil resource patches [7,48–51]. In this later case, plants may not sense soil heterogeneity and thus fail to show any response. Even though we did not measure the growth of each component plant species, thus failing to evaluate the pair-wise plant interaction, we found that the three studied functional groups differed in their responses to soil heterogeneity. The growth of forbs was largely reduced in heterogeneous soils, but it played a limited role in the growth of the whole community due to its extremely low productivity. By contrast, we found that the two predominant functional groups (i.e., grasses and legumes: biomass ratios >90%) produced more biomass in the high than in the low nutrient soil patches within the heterogeneous soil. This indicated that these two plant groups responded to soil nutrient heterogeneity in our study. These differences were caused by the reduced growth of legumes in the low nutrient soil patches, and the increased growth of grasses in the high nutrient soil patches. Therefore, the overall growth was not promoted in the heterogeneous soils compared with that in the homogeneous soils. These results indicate that soil nutrient heterogeneity can influence growth of the studied plant functional groups within a community through changing their localized responses to the quality of soil patches within the heterogeneous soil [7,48,49]. These responses, however, may not have a cascade effect on the community-level productivity due to the counteraction of positive and negative effects [52].

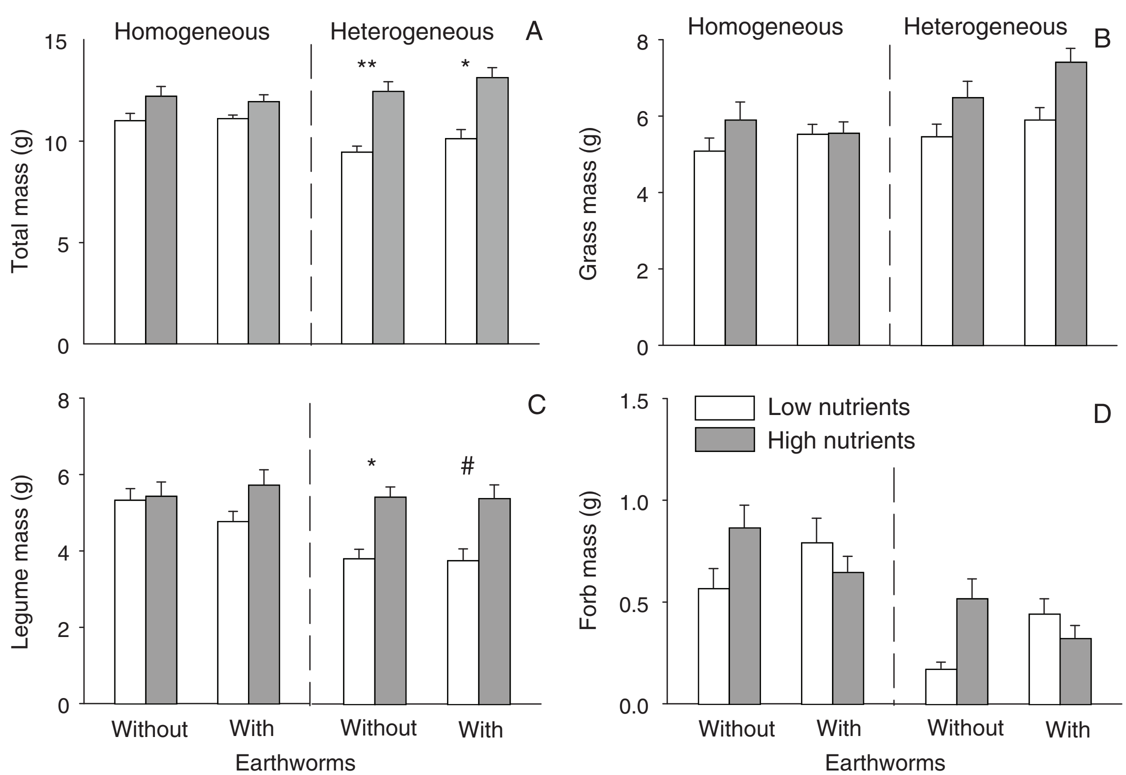

Figure 4: (A) Total aboveground biomass of the communities and (B–D) aboveground biomass of the three functional groups. Means + 1 SE are shown. Symbols (**$p < 0.01$, *$p < 0.05$ and $^{\#}p < 0.1$) indicate significant differences between the low and high nutrient soil patches within homogeneous and heterogeneous soils within each earthworm treatment

Table 2: Results of repeated-measures ANOVAs for the effects of soil nutrient heterogeneity, earthworms and patch quality on total aboveground biomass of the community, and aboveground biomass and aboveground biomass ratio of each functional group

Effect	Total mass	Grass mass	Legume mass	Forb mass	Grass mass ratio	Legume mass ratio	Forb mass ratio
Between-subject							
Heterogeneity (H)	0.80^{ns}	1.74^{ns}	2.40^{ns}	**9.00***	*2.88*$^{\#}$	1.55^{ns}	**9.57***
Earthworm (E)	0.60^{ns}	0.54^{ns}	0.09^{ns}	0.24^{ns}	0.36^{ns}	0.52^{ns}	0.30^{ns}
H × E	0.63^{ns}	0.19^{ns}	0^{ns}	0.07^{ns}	0.03^{ns}	0.08^{ns}	0.06^{ns}
Within-subject							
Patch quality (P)	**9.65***	4.01$^{\#}$	**10.61***	1.47^{ns}	1.51^{ns}	1.59^{ns}	0.46^{ns}
P × H	2.54^{ns}	1.15^{ns}	*3.40*$^{\#}$	0.01^{ns}	0.71^{ns}	1.16^{ns}	0.08^{ns}
P × E	0.01^{ns}	0.02^{ns}	0.37^{ns}	*3.85*$^{\#}$	<0.01^{ns}	1.49^{ns}	**4.94***
P × H × E	0.03^{ns}	0.67^{ns}	0.41^{ns}	0.11^{ns}	1.47^{ns}	1.54^{ns}	0.17^{ns}

Note: F and significant level values ($^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $^{\#}p < 0.1$; $^{ns}p > 0.1$) are shown. Degree of freedom was 1, 34 for all the effects. Data were root-squaredly transformed.

We did not find evidence that earthworms can promote plant community productivity, despite many studies have reported positive effects of earthworms on the growth of single plant species [38,53]. Earthworms can increase soil nutrient availability which is essential for plant growth [54]. However, such positive effects may not be sufficient to promote growth of the plant community [55]. Unfortunately, in the present study, we did not know exactly whether the presence of earthworms increased soil nutrient availability, and how this process may relate to growth of the plant community. Alternatively, pot cultures are generally far from optimal to support life of earthworms, which may lead to their death [26,56,57]. This was likely the case in our study, as we observed only a small number of living earthworms at the end of the experiment. Moreover, these living earthworms may have rather limited influences on soil processes and plant growth due to the short experimental duration. Therefore, future studies should test the relationships between earthworms, soils and plants in long-term experiments under field conditions where earthworms prevail.

We expected that the presence of earthworms may alter the effectiveness of soil nutrient heterogeneity on plant community performance. We found that the presence of earthworms changed the response of forbs to soil patch quality within the heterogeneous soil probably due to their distinct rooting system. However, their presence did not change the growth response of the two dominant functional groups (grasses and legumes) to soil heterogeneity. This was indicated by the observation that there was a clear growth difference in the two dominant groups between the low and high nutrient soil patches within the heterogeneous soil. However, there was a similar growth between these two (imagined) types of patches within the homogeneous soil, independently of the presence of earthworms. Therefore, the extremely weak effects of earthworms on the dominant functional groups in our study may have failed to change the heterogeneity effects at the plant community level, probably due to a lower survival rate of the earthworms [26,56,57].

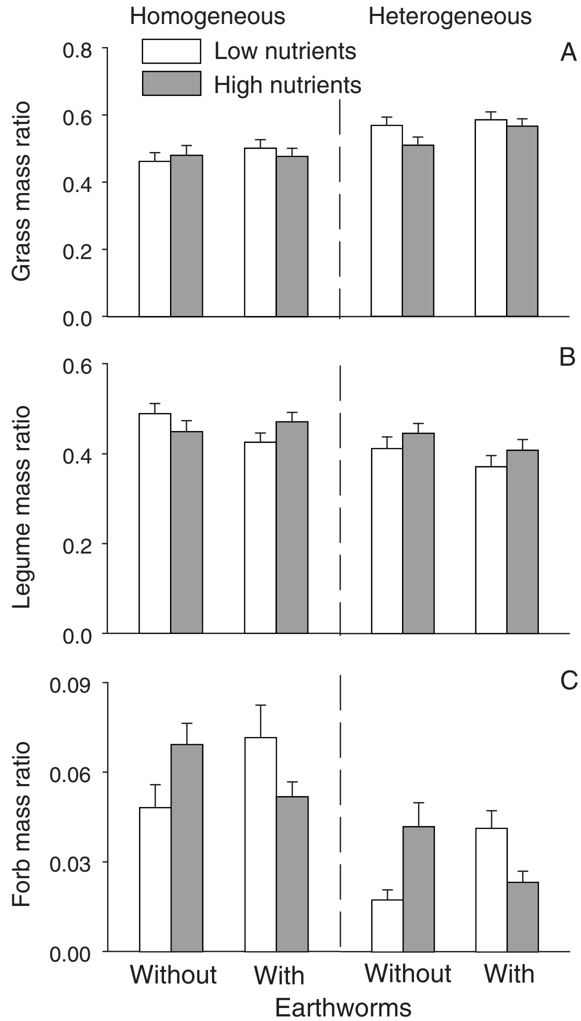

Figure 5: Aboveground biomass ratio of the (A) grasses, (B) legumes and (C) forbs. Means + 1 SE are shown

In conclusion, our findings do not support the idea that soil nutrient heterogeneity and earthworms can promote plant community productivity. However, soil patch quality within the heterogeneous soil influenced plant community productivity through changing growth of the component plant functional groups. Moreover, these effects varied depending on the functional group identity and the presence of earthworms. However, the results of this short-term study were obtained under artificial soil conditions. Short-term community responses to artificial soil heterogeneity and earthworms in terms of aboveground biomass, as found in our study, may not be comparable to long-term studies at the field. This might be because characteristics of both plant communities and earthworm populations may change over time under different soil conditions due to complex plant-soil-earthworm interactions [58–60]. Despite this, our results highlight the importance of ecological effects of soil nutrient heterogeneity on plant community performance. Future studies should unravel the underlying mechanisms of soil nutrient heterogeneity and their potential interaction with earthworms under more realistic conditions.

Funding Statement: This work was supported by the National Natural Science Foundation of China (Grant No. 31800341).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Petrenko, M., Friedman, S. P., Fluss, R., Pasternak, Z., Huppert, A. et al. (2020). Spatial heterogeneity stabilizes predator–prey interactions at the microscale while patch connectivity controls their outcome. *Environmental Microbiology, 22(2),* 694–704. DOI 10.1111/1462-2920.14887.
2. Liu, J., Zhu, X. W., Yu, F. H., Dong, M., Zhang, S. M. et al. (2003). Spatial heterogeneity of *Ulmus pumila* open forest ecosystem in Otindag sandy land. *Environmental Science, 24(4),* 29–34.
3. Liang, S. C., Zhang, S. M., Yu, F. H., Dong, M. (2007). Small-scale spatial cross-correlation between ramet population variables of *Potentilla reptans* Var. *sericophylla* and soil available phosphorus. *Chinese Journal of Plant Ecology, 31(5),* 613–618. DOI 10.17521/cjpe.2007.0113.
4. Day, K. J., John, E. A., Hutchings, M. J. (2003). The effects of spatially heterogeneous nutrient supply on yield, intensity of competition and root placement patterns in *Briza media* and *Festuca ovina*. *Functional Ecology, 17(4),* 454–463. DOI 10.1046/j.1365-2435.2003.00758.x.
5. Hutchings, M. J., John, E. A. (2004). The effects of environmental heterogeneity on root growth and root/shoot partitioning. *Annals of Botany, 94(1),* 1–8. DOI 10.1093/aob/mch111.
6. Fransen, B., de Kroon, H., Berendse, F. (2001). Soil nutrient heterogeneity alters competition between two perennial grass species. *Ecology, 82(9),* 2534–2546. DOI 10.1890/0012-9658(2001)082[2534:SNHACB]2.0.CO;2.
7. Bliss, K. M., Jones, R. H., Mitchell, R. J., Mou, P. P. (2002). Are competitive interactions influenced by spatial nutrient heterogeneity and root foraging behavior? *New Phytologist, 154(2),* 409–417. DOI 10.1046/j.1469-8137.2002.00389.x.
8. Xue, W., Huang, L., Yu, F. H. (2020). Importance of starting points in heterogeneous environments: Interactions between two clonal plants with contrasting spatial architectures. *Journal of Plant Ecology, 13(3),* 323–330. DOI 10.1093/jpe/rtaa018.
9. Xue, W., Huang, L., Yu, F. H. (2021). Increasing soil configurational heterogeneity promotes plant community evenness through equalizing differences in competitive ability. *Science of the Total Environment, 750,* 142308. DOI 10.1016/j.scitotenv.2020.142308.
10. Zhou, J., Dong, B. C., Alpert, P., Li, H. L., Zhang, M. X. et al. (2012). Effects of soil nutrient heterogeneity on intraspecific competition in the invasive, clonal plant *Alternanthera philoxeroides*. *Annals of Botany, 109(4),* 813–818. DOI 10.1093/aob/mcr314.
11. Peng, Y. K., Luo, F. L., Li, H. L., Yu, F. H. (2013). Growth responses of a rhizomatous herb *Bolboschoenus planiculmis* to scale and contrast of soil nutrient heterogeneity. *Chinese Journal of Plant Ecology, 37(4),* 335–343. DOI 10.3724/SP.J.1258.2013.00033.
12. Dong, B. C., Wang, J. Z., Liu, R. H., Zhang, M. X., Luo, F. L. et al. (2015). Soil heterogeneity affects ramet placement of *Hydrocotyle vulgaris*. *Journal of Plant Ecology, 8(1),* 91–100. DOI 10.1093/jpe/rtu003.
13. Birch, C. P. D., Hutchings, M. J. (1994). Exploitation of patchily distributed soil resources by the clonal herb *Glechoma hederacea*. *Journal of Ecology, 82(3),* 653–664. DOI 10.2307/2261272.
14. Wijesinghe, D. K., Hutchings, M. J. (1997). The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: An experimental study with *Glechoma hederacea*. *Journal of Ecology, 85(1),* 17–28. DOI 10.2307/2960624.
15. Wijesinghe, D. K., Hutchings, M. J. (1999). The effects of environmental heterogeneity on the performance of *Glechoma hederacea*: The interactions between patch contrast and patch scale. *Journal of Ecology, 87(5),* 860–872. DOI 10.1046/j.1365-2745.1999.00395.x.
16. Loreau, M., Mouquet, N., Gonzalez, A. (2003). Biodiversity as spatial insurance in heterogeneous landscapes. *Proceedings of the National Academy of Sciences of the United States of America, 100(22),* 12765–12770. DOI 10.1073/pnas.2235465100.

17. Wijesinghe, D. K., John, E. A., Hutchings, M. J. (2005). Does pattern of soil resource heterogeneity determine plant community structure? An experimental investigation. *Journal of Ecology, 93(1),* 99–112. DOI 10.1111/j.0022-0477.2004.00934.x.
18. Fitter, A. H. (1982). Influence of soil heterogeneity on the coexistence of grassland species. *Journal of Ecology, 70(1),* 139–148. DOI 10.2307/2259869.
19. Wacker, L., Baudois, O., Eichenberger-Glinz, S., Schmid, B. (2008). Environmental heterogeneity increases complementarity in experimental grassland communities. *Basic and Applied Ecology, 9(5),* 467–474. DOI 10.1016/j.baae.2007.08.003.
20. Costanza, J. K., Moody, A., Peet, R. K. (2011). Multi-scale environmental heterogeneity as a predictor of plant species richness. *Landscape Ecology, 26(6),* 851–864. DOI 10.1007/s10980-011-9613-3.
21. Decaëns, T., Jiménez, J. J., Lavelle, P. (1999). Effect of exclusion of the anecic earthworm *Martiodrilus carimaguensis* Jimenez and Moreno on soil properties and plant growth in grasslands of the eastern plains of Colombia. *Pedobiologia, 43(6),* 835–841.
22. Sánchez-de León, Y., Lugo-Pérez, J., Wise, D. H., Jastrow, J. D., González-Meler, M. A. (2014). Aggregate formation and carbon sequestration by earthworms in soil from a temperate forest exposed to elevated atmospheric CO_2: A microcosm experiment. *Soil Biology and Biochemistry, 68,* 223–230. DOI 10.1016/j.soilbio.2013.09.023.
23. Nieminen, M., Hurme, T., Mikola, J., Regina, K., Nuutinen, V. (2015). Impact of earthworm *Lumbricus terrestris* living sites on the greenhouse gas balance of no-till arable soil. *Biogeosciences, 12(18),* 5481–5493. DOI 10.5194/bg-12-5481-2015.
24. Hoang, D. T., Razavi, B. S., Kuzyakov, Y., Blagodatskaya, E. (2016). Earthworm burrows: Kinetics and spatial distribution of enzymes of C-, N- and P-cycles. *Soil Biology and Biochemistry, 99(8),* 94–103. DOI 10.1016/j.soilbio.2016.04.021.
25. Shuster, W. D., Subler, S., McCoy, E. (2001). Deep-burrowing earthworm additions changed the distribution of soil organic carbon in a chisel-tilled soil. *Soil Biology and Biochemistry, 33(7–8),* 983–996. DOI 10.1016/S0038-0717(01)00002-5.
26. Fischer, C., Roscher, C., Jensen, B., Eisenhauer, N., Baade, J. et al. (2014). How do earthworms, soil texture and plant composition affect infiltration along an experimental plant diversity gradient in grassland? *PLoS One, 9(6),* e98987. DOI 10.1371/journal.pone.0098987.
27. Laossi, K. R., Ginot, A., Noguera, D. C., Blouin, M., Barot, S. (2010). Earthworm effects on plant growth do not necessarily decrease with soil fertility. *Plant and Soil, 328(1–2),* 109–118. DOI 10.1007/s11104-009-0086-y.
28. Eisenhauer, N., Butenschoen, O., Radsick, S., Scheu, S. (2010). Earthworms as seedling predators: Importance of seeds and seedlings for earthworm nutrition. *Soil Biology and Biochemistry, 42(8),* 1245–1252. DOI 10.1016/j.soilbio.2010.04.012.
29. Forey, E., Barot, S., Decaëns, T., Langlois, E., Laossi, K. R. et al. (2011). Importance of earthworm–seed interactions for the composition and structure of plant communities: A review. *Acta Oecologica, 37(6),* 594–603. DOI 10.1016/j.actao.2011.03.001.
30. Clause, J., Barot, S., Forey, E. (2016). Earthworms promote greater richness and abundance in the emergence of plant species across a grassland-forest ecotone. *Journal of Plant Ecology, 9(6),* 703–711. DOI 10.1093/jpe/rtw008.
31. Ayanlaja, S., Owa, S., Adigun, M., Senjobi, B., Olaleye, A. (2001). Leachate from earthworm castings breaks seed dormancy and preferentially promotes radicle growth in jute. *Hortscience, 36(1),* 143–144. DOI 10.21273/HORTSCI.36.1.143.
32. Blouin, M., Zuily-Fodil, Y., Pham-Thi, A. T., Laffray, D., Reversat, G. et al. (2005). Belowground organism activities affect plant aboveground phenotype, inducing plant tolerance to parasites. *Ecology Letters, 8(2),* 202–208. DOI 10.1111/j.1461-0248.2004.00711.x.
33. Araujo, Y., Luizão, F. J., Barros, E. (2004). Effect of earthworm addition on soil nitrogen availability, microbial biomass and litter decomposition in mesocosms. *Biology and Fertility of Soils, 39(3),* 146–152. DOI 10.1007/s00374-003-0696-0.

34. Knowles, M. E., Ross, D. S., Görres, J. H. (2016). Effect of the endogeic earthworm *Aporrectodea tuberculata* on aggregation and carbon redistribution in uninvaded forest soil columns. *Soil Biology and Biochemistry, 100,* 192–200. DOI 10.1016/j.soilbio.2016.06.016.
35. Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M. et al. (2015). Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? *Soil and Tillage Research, 146,* 118–124. DOI 10.1016/j.still.2014.01.007.
36. Angst, Š., Mueller, C. W., Cajthaml, T., Angst, G., Lhotáková, Z. et al. (2017). Stabilization of soil organic matter by earthworms is connected with physical protection rather than with chemical changes of organic matter. *Geoderma, 289(5),* 29–35. DOI 10.1016/j.geoderma.2016.11.017.
37. Frouz, J. (2017). Effects of soil development time and litter quality on soil carbon sequestration: Assessing soil carbon saturation with a field transplant experiment along a post-mining chronosequence. *Land Degradation & Development, 28(2),* 664–672. DOI 10.1002/ldr.2580.
38. Liu, L., Alpert, P., Dong, B. C., Yu, F. H. (2020). Modification by earthworms of effects of soil heterogeneity and root foraging in eight species of grass. *Science of the Total Environment, 708,* 134941. DOI 10.1016/j.scitotenv.2019.134941.
39. McLaren, J. R., Turkington, R. (2010). Ecosystem properties determined by plant functional group identity. *Journal of Ecology, 98(2),* 459–469. DOI 10.1111/j.1365-2745.2009.01630.x.
40. Chen, J. R., Wu, Z. Y., Raven, P. H. (1999). *Flora of China*. Beijing: Science Press.
41. Gunadi, B., Edwards, C. A. (2003). The effects of multiple applications of different organic wastes on the growth, fecundity and survival of *Eisenia fetida* (Savigny)(Lumbricidae). *Pedobiologia, 47(4),* 321–329. DOI 10.1078/0031-4056-00196.
42. Aira, M., Monroy, F., Domínguez, J. (2006). *Eisenia fetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. *Microbial Ecology, 52(4),* 738–747. DOI 10.1007/s00248-006-9109-x.
43. Cao, Z., Qiao, Y., Wang, B., Qin, X. (2006). Influence of agricultural intensification on the earthworm community in arable farmland in the North China Plain. *European Journal of Soil Biology, 42,* S362–S366. DOI 10.1016/j.ejsobi.2006.07.014.
44. Smith, R. G., McSwiney, C. P., Grandy, A. S., Suwanwaree, P., Snider, R. M. et al. (2008). Diversity and abundance of earthworms across an agricultural land-use intensity gradient. *Soil and Tillage Research, 100(1–2),* 83–88. DOI 10.1016/j.still.2008.04.009.
45. Tilman, D. (1982). *Resource competition and community structure*. Princeton University Press, Princeton.
46. Levine, J. M., HilleRisLambers, J. (2009). The importance of niches for the maintenance of species diversity. *Nature, 461(7261),* 254–257. DOI 10.1038/nature08251.
47. Wilson, J. B. (2011). The twelve theories of co-existence in plant communities: The doubtful, the important and the unexplored. *Journal of Vegetation Science, 22(1),* 184–195. DOI 10.1111/j.1654-1103.2010.01226.x.
48. Wijesinghe, D. K., John, E. A., Beurskens, S., Hutchings, M. J. (2001). Root system size and precision in nutrient foraging: Responses to spatial pattern of nutrient supply in six herbaceous species. *Journal of Ecology, 89(6),* 972–983. DOI 10.1111/j.1365-2745.2001.00618.x.
49. Mommer, L., Visser, E. J., van Ruijven, J., de Caluwe, H., Pierik, R. et al. (2011). Contrasting root behaviour in two grass species: A test of functionality in dynamic heterogeneous conditions. *Plant and Soil, 344(1–2),* 347–360. DOI 10.1007/s11104-011-0752-8.
50. Liu, Y., Bortier, M. F., de Boeck, H. J., Nijs, I. (2017). Root distribution responses to three-dimensional soil heterogeneity in experimental mesocosms. *Plant and Soil, 421(1),* 353–366. DOI 10.1007/s11104-017-3472-x.
51. Stover, H. J., Henry, H. A. L. (2018). Soil homogenization and microedges: Perspectives on soil-based drivers of plant diversity and ecosystem processes. *Ecosphere, 9(6),* e02289. DOI 10.1002/ecs2.2289.
52. Casper, B. B., Cahill, J. F. (1996). Limited effects of soil nutrient heterogeneity on populations of *Abutilon theophrasti* (Malvaceae). *American Journal of Botany, 83(3),* 333–341. DOI 10.1002/j.1537-2197.1996.tb12714.x.

53. Noguera, D., Rondón, M., Laossi, K. R., Hoyos, V., Lavelle, P. et al. (2010). Contrasted effect of biochar and earthworms on rice growth and resource allocation in different soils. *Soil Biology and Biochemistry, 42(7),* 1017–1027. DOI 10.1016/j.soilbio.2010.03.001.
54. Shuster, W. D., Subler, S., McCoy, E. L. (2001). Deep-burrowing earthworm additions changed the distribution of soil organic carbon in a chisel-tilled soil. *Soil Biology and Biochemistry, 33(7–8),* 983–996. DOI 10.1016/S0038-0717(01)00002-5.
55. Zaller, J. G., Arnone, J. A. III (1999). Earthworm and soil moisture effects on the productivity and structure of grassland communities. *Soil Biology and Biochemistry, 31(4),* 517–523. DOI 10.1016/S0038-0717(98)00126-6.
56. van Rhee, J. A. (1965). Earthworm activity and plant growth in artificial cultures. *Plant and Soil, 22(1),* 45–48. DOI 10.1007/BF01377688.
57. Edwards, C. A., Bohlen, P. J. (1996). *Biology and ecology of earthworms*. Chapman and Hall, London.
58. Baer, S. G., Collins, S. L., Blair, J. M., Knapp, A. K., Fiedler, A. K. (2005). Soil heterogeneity effects on tallgrass prairie community heterogeneity: An application of ecological theory to restoration ecology. *Restoration Ecology, 13(2),* 413–424. DOI 10.1111/j.1526-100X.2005.00051.x.
59. Baer, S. G., Blair, J. M., Collins, S. L. (2016). Environmental heterogeneity has a weak effect on diversity during community assembly in tallgrass prairie. *Ecological Monographs, 86(1),* 94–106. DOI 10.1890/15-0888.1.
60. Xue, W., Bezemer, T. M., Berendse, F. (2019). Soil heterogeneity and plant species diversity in experimental grassland communities: Contrasting effects of soil nutrients and pH at different spatial scales. *Plant and Soil, 442(1–2),* 497–509. DOI 10.1007/s11104-019-04208-5.