Introducing the anecic earthworm *Lumbricus terrestris* in grasslands to improve water regulation

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Abstract

Water regulation is an important ecosystem service of grasslands. Anecic earthworm *Lumbricus terrestris* presence in grasslands has a positive effect on water infiltration. We explored the ability of *L. terrestris* to survive and reproduce after being newly introduced into mesocosms in grasslands on sandy soils. While *L. terrestris* appeared able to survive and produce cocoons, survival rate was low (32% after 7 months, 6% after 15 months, 33% after 8 months) and the number of juveniles was low (2.6 and 2.7 ind. m^{-2} after 7 and 15 months, resp., 2.5 ind. m^{-2} after 8 months in re-inoculated mesocosms). Low survival rate may be related to the life history of the *L. terrestris* inoculum, soil moisture, interspecific competition for food with the native population of epigeic earthworms and the risk of predation.

Keywords: earthworm inoculation; mesocosm; water regulation; interspecific competition

Introduction

Grasslands play a vital role in water regulation. Global climate changes are characterised by prolonged dry periods and intensified peak rainfall (Pachauri *et al.*, 2014). Both entail major impacts on agricultural grasslands (Beier *et al*., 2012). As soil ecosystem engineers, earthworms cause soil bioturbation which helps to improve water infiltration (Deru *et al*., 2018). Deep-burrowing earthworms such as *Lumbricus terrestris* create vertical, semi-permanent burrows, reaching down to 2 m. Burrows can increase the soil's infiltration rate and capacity, (Blouin *et al.*, 2013), while also increasing rooting space, which promotes drought tolerance (Edwards *et al.*, 1980). *L. terrestris* is currently present at 20-25% of Dutch dairy farms on sandy soil. It is likely that water infiltration in grasslands where *L. terrestris* is absent could be improved through *L. terrestris* burrowing activity. As natural dispersal is slow and can be hampered by obstacles (roads, waterways etc.), we explored the possibility of inoculating grassland with this species. We hypothesised that *L. terrestris* would be able to survive and reproduce, but would possibly suffer from interspecific competition with the resident earthworm population. Additionally, that loosening the soil and removing the resident earthworm population prior to *L. terrestris* inoculation increase survival and reproduction rate, as the earthworms would benefit from easier burrowing and less competition.

Materials and methods

In April 2019, 40 mesocosms (steel pipes of 61 cm diameter, 50 cm in length) were driven 40 cm into the soil in permanent grasslands on sandy soil at two Dutch dairy farms. Twenty mesocosms per grassland, in two rows, 90 cm apart. Farm A was a conventional farm with a history of artificial fertiliser and slurry application, farm B an organic farm with a history of farmyard manure use (for details see Van de Logt *et al*., 2023). Per farm, half of the mesocosms were inoculated with adult Canadian *L. terrestris* 52 (ind. m^{-2}) (purchased commercially), the other mesocosms served as controls. All mesocosms were covered with a net and mowed regularly. Escape belowground was highly unlikely, as *L. terrestris* burrows vertically and disperses over the soil surface (Mather and Christensen, 1988). After 7 months, in November 2019, half of the mesocosms were harvested at both locations. The soil from each mesocosm was removed in three layers (0–20, 20–40 and 40–60 cm) and hand-sorted to collect all earthworms. Earthworms were conserved for species determination. The hand-sorted soil layers were returned to the mesocosms in their original order (hand-sorting had loosened the soil and made it devoid of earthworms, but with cocoons still present), and reseeded with grass-clover. The harvested mesocosms were re-inoculated with the same amount of *L. terrestris* as April 2019. Fifteen months after initial installation, in July 2020, the soil of all mesocosms was harvested following the same procedure as in November. ANOVA in GenStat with location (A, B), treatment (control, inoculated) and harvesting date (November, July) as factors was used to analyse the data.

Results and discussion

Of the *L. terrestris* that were introduced in April 2019, 32% (16.1 ind. m^{-2}) had persisted after 7 months and 6% (2.9 ind. m^{-2}) after 15 months. In the mesocosms that were re-inoculated in November 2019, 33% (16.9 ind. m^{-2}) of the *L. terrestris* had persisted (Figure 1). We assumed all adult *L. terrestris* to be introduced individuals, as it can take them over one year to mature under field conditions (Daniel, 1992). Recovery 7-8 months post-introduction was comparable to earlier findings by Andriuzzi *et al.* (2015).

Loosened soil and the initial absence of a resident earthworm population (the population largely recovered over the period of the experiment) had not resulted in high survival nor reproduction rate. However, as the 7- and 8-month experiments did not run parallel in time, we cannot draw a final conclusion on the effect of introduction into hand-sorted soil.

We propose several factors that may have contributed to this low survival rate. First, as *L. terrestris* is not bred commercially, we only had access to earthworms imported from Canada. The weeks of inactivity due to the import procedure may have weakened them. It is also possible that the Dutch grassland hosted pathogens for the earthworms. Second, the spring of 2020 was exceptionally dry. In general, *L. terrestris* remains active during periods of drought (Eisenhauer *et al.*, 2008), which may make the species more susceptible to the potentially lethal effects of low soil moisture. Third, *L. terrestris* may have suffered from interspecific competition for food with epigeic earthworms, mainly *Lumbricus rubellus*, as this species also feeds on surface organic matter, but has a higher growth and reproduction rate, *L. rubellus* was the most abundant epigeic species for both locations (99% at location A and 84% at location B). The difference was non-significant but, on average, the inoculated mesocosms contained fewer epigeic worms than the control mesocosms at both locations and both harvesting dates. The discovery of juveniles showed that *L. terrestris* had successfully produced cocoons after introduction. However, it is unclear that the actual mating took place in the mesocosms, as the species can store sperm for several months. Fourth, small

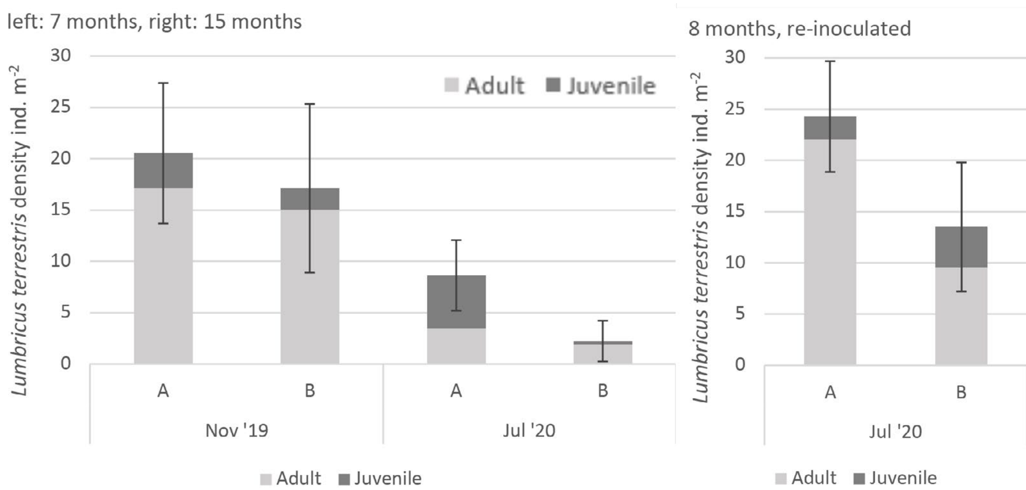

Figure 1. *L. terrestris* density at farm A and farm B after 7 months (two left bars) and 15 months (two middle bars). The two bars on the right side present *L.* density after 8 months in re-inoculated mesocosms.

holes were observed in some of the protective nets, these may be signs of attempted predation by birds. Therefore, we cannot exclude the possibility that some earthworms had been predated on.

Conclusion

L. terrestris can survive for 15 months and produce cocoons after introduction into grassland on sandy soil in a mesocosm set-up. The life history of *L. terrestris*, a lack of soil moisture, interspecific competition and predation could all threaten the inoculated earthworms. Despite these challenges, a number of individuals managed to survive and produce cocoons, showing certain potential for *L. terrestris* inoculation as an ecological innovation towards climate adaptation in agricultural grasslands. However, as survival rate was low, only further experimental trials over greater time spans and in non-enclosed plots will determine whether there is realistic potential for *L. terrestris* to develop a stable population.

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