Gunnera tinctoria invasions increase, not decrease, earthworm abundance and diversity

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Abstract Invasive plants often modify soil biotic communities through changes in soil physicochemical characteristics or the amount and/or quality of litter inputs. We assessed the impacts of *Gunnera tinctoria* invasions on soil and the earthworm community, on Achill Island, Co. Mayo, Ireland. We compared replicated (n = 5) areas invaded by *G. tinctoria* with uninvaded semi-natural grasslands, as well as with areas subjected to mechanical removal or herbicide treatment. Modifications in physiochemical properties included lower soil temperatures and higher soil pH during the summer in invaded areas, yet little effect on C and N stocks, or soil moisture.

Marked differences in litter were observed, however, with invaded areas having *c.* 20-fold higher (aboveground) litter input than uninvaded ones, as well as lower C:N ratio (17 vs. 29). This was associated with a significantly higher overall abundance and biomass of earthworms in invaded plots (375 individuals m^{-2}, 115 g biomass m^{-2}), compared to the uninvaded control (130 individuals m^{-2}, 45 g biomass m^{-2}), with removal treatments having intermediate values. Earthworm communities comprised 10 species, typical for Irish grasslands, dominated by the common endogeic species *Allolobophora chlorotica, Aporrectodea caliginosa* and *Aporrectodea rosea*. Both earthworm species richness and Shannon diversity were significantly higher in invaded areas, but only in spring samples. Based on this new information, plant invaders may increase the abundance and diversity of earthworms, mainly due to much larger litter inputs, increased soil pH and possibly lower soil temperatures in the summer.typical of Irish grasslands

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Introduction

Plant invasions have the potential to alter both the structure and function of a range of ecosystems and the services they provide (Vitousek et al. 1997; Ehrenfeld 2010; Ricardi et al. 2017). To date,

however, most studies that have examined the effect of plant invasions on terrestrial ecosystems have focused on above-ground changes and how these impact on vegetation productivity and plant biodiversity (Ehrenfeld. 2010; Vilà et al. 2011). Plant invasions are also recognised to influence soil chemical properties (Ehrenfeld 2003), modifying biogeochemical cycling, and impacting on C and N stocks. Nevertheless, there is a lack of detailed quantitative information on the time dependence of these effects of invasive plants (D'Antonio and Flory 2017), with some impacts disappearing with time.

Whilst plant invasions can also affect the soil biota, much less information is available. A range of largely case-specific effects have been described that vary both in the direction and magnitude of any impact (e.g., Belnap and Phillips 2001; Kourtev et al. 2003; Callaway et al. 2004; Vilà et al. 2011; Simberloff et al. 2013; Meisner et al. 2014). Clearly, however, there are close interactions between any changes in soil properties and the soil biota. Not only will any changes in plant-invasion-related soil biogeochemical properties influence the soil biota, but their influence on soil structure and decomposition processes will, in turn, also influence biogeochemical cycling. Of relevance, as far as the impacts of alien invasions are concerned, is whether this chain of effects (i.e., invasion meltdown; Simberloff and Von Holle 1999) is always detrimental with consistent effects on individuals or processes.

The available evidence suggests that a range of soil biotic responses have been reported, including detrimental, beneficial, or neutral effects (Simberloff et al. 2013; Guerin et al. 2017; Kuebbing and Nunez 2018). Despite evidence for biological invasions having an overall detrimental impact with significant economic costs (Diagne et al. 2021), a more complete assessment will require detailed information on a range of organisms and ecosystem processes that also consider both detrimental and beneficial effects (Vimercati et al. 2020). Any impact is also likely to be context dependent, so an individual species or a trait purported to be associated with invasiveness might have contrasting effects depending on site, location, the extent of human activity or the prevailing environmental conditions (Sapsford et al. 2020). Only through an understanding of the impacts of plant invasions on multiple targets/processes and trophic levels will we be able to provide a comprehensive assessment of the totality of their effects and what the implications are for management and restoration purposes (Vimercati et al. 2020). Importantly, the impact may vary with trophic level, with some trophic levels more vulnerable than others (Bradley et al. 2016; Schirmel et al. 2016), as well as being ecosystem specific (McCary et al. 2016).

Earthworms are recognised as one of the most important components of the soil biota in many terrestrial ecosystems (Curry 1994), chiefly through their direct contributions to organic matter decomposition, nitrogen mineralisation, and the maintenance of soil structure, giving them key roles in the cycling of nutrients and water (Blouin et al. 2013). In addition, earthworms can affect many soil processes indirectly through their impact on microbial communities (Le Bayon et al. 2017). Through their contribution to litter decomposition, earthworms provide a route for the incorporation of organic matter into the soil, as well as its subsequent stabilisation by the formation of organo-mineral aggregates (Blouin et al. 2013). Litter bag experiments have shown a strong correlation between litter decomposition and earthworm numbers, although this also depends on litter palatability, with earthworms often thought to prefer litter with a low polyphenol content (Hendriksen 1990; Curry and Schmidt 2007). However, this could depend on the concentration and composition of individual polyphenols, as well as the possibility that litter palatability could be influenced by variations in litter quality, as measured by changes in the C:N ratio (Hendriksen 1990). In the context of plant invasions this means that litter palatability may also be important despite the generally higher litter production associated with many plant invaders (Ehrenfeld 2003; Wolkovich et al. 2010; Tamura and Tharayil 2014).

Earthworms (Lumbricidae) can be divided into three functional groups (Bouché 1972), and their relative abundance will determine, to a large extent, how they influence different soil-based processes. Epigeic earthworms live on or near the soil surface and feed directly on accumulated litter; endogeic earthworms live in the upper mineral soil horizons producing temporary horizontal burrows and feeding on mineral soil and partly decomposed material; the last group, anecic earthworms, produces permanent vertical burrows and feeds on surface litter dragged into their burrows (Bouché 1972). Although plant invasions could affect all three groups in the long term, the generally higher

litter production could provide a shorter-term trophic benefit for epigeic or anecic earthworms. Earthworms may also feed on dead and decaying roots and root exudates, so the supply of this food source might be important too but is much less understood (Curry and Schmidt 2007; Gaudon et al. 2020). Modifications in a range of physicochemical soil factors (e.g., water and temperature dynamics, pH, Ca concentrations) associated with plant invasions (Ehrenfeld 2003; 2010) could also influence the abundance and biodiversity of soil earthworm populations. Differences in the plant species composition between invaded and uninvaded areas may be important too, given the often close relationship between earthworm abundance and vegetation type (Singh et al. 2016).

Invasions by the unusual, N-fixing, herbaceous species *Gunnera tinctoria* (Osborne et al. 1991) represents a major aspect of land-cover change with estimates suggesting a > 10-fold increase in the area occupied in Ireland over the last 50 years (Fennell et al. 2014). As well as being a significant invader in Ireland, this species has become invasive in other parts of western Europe, the UK, Portugal, and New Zealand (Osborne et al. 1991; Gioria and Osborne 2013). Several aspects of these invasions in Ireland have already been documented, including impacts on the soil seed bank (Gioria and Osborne 2009; 2010; Gioria et al. 2011), the significance of differences in N-utilisation and phenology (Mantoani et al. 2020) and effects on soil greenhouse gas emissions (Mantoani and Osborne 2021). Importantly, experimental evidence, supported by biological records (Fennell et al. 2014), have allowed us to date the age of the populations investigated at > 50 years, which enables us to document the time over which any changes have occurred. Although these invasions are associated with the production of a large amount of litter compared to uninvaded areas (Gioria and Osborne 2013; Mantoani 2019) there was surprisingly little evidence of any significant changes in C or N stocks, despite the age (>50 years) of the invasion (neutral impact; Mantoani 2019), whilst soil CO_2 emissions were significantly reduced (beneficial impact; Mantoani and Osborne 2021). Conversely, detrimental effects on above-ground vegetation productivity and composition, together with reductions in seed bank abundance and diversity have been reported (Gioria and Osborne 2009; 2010; 2013; Gioria et al. 2011; Mantoani and Osborne, 2022), demonstrating the contrasting

responses that can be observed, depending on the focus or target of the investigation.

To extend and complement these studies, we examined the effect of *G. tinctoria* invasions on native earthworm populations, considering the three functional earthworm groups, as well as earthworm species and community diversity. Given the potential legacy effects associated with plant invasions, we also assessed the impact of the experimental removal of *G. tinctoria* on earthworm populations using physical or chemical (herbicide) treatments. We also assessed the effects changes in litter accumulation and quality and the extent to which this may have influenced earthworm communities in both invaded and uninvaded areas. Our hypotheses were: (1) *G. tinctoria* invasions would, because of the production of an unpalatable litter with a high polyphenol content (Zamorano et al. 2017), result in a litter-quality related reduction in soil earthworm populations (Hendriksen 1990); and (2) there would be a legacy effect of *G. tinctoria* invasions with reduced earthworm populations in areas where *G. tinctoria* plants were removed physically or chemically.

Material and Methods

Area of study and experimental design

The study area is the same as that reported in Mantoani et al. (2020) and Mantoani and Osborne (2021; 2022), and located on Dooega Beach, Achill Island (53º51" and 54º01' N; and 9º55' and 10º15' W), Co. Mayo, on the west coast of Ireland. We evaluated the earthworm populations in four treatments: GRASS, uninvaded semi-natural grasslands, GUN, areas invaded by *G. tinctoria*, and two *G. tinctoria* removal treatments, MER, where plants were subjected mechanical removal, and HER, herbicide treatment (RoundUp Flex, Monsanto ® - concentration 4 L ha^{-1}). For the experiments, we selected five fields (minimum size of 1 hectare each) and randomly assigned four replicate plots (5 × 5 m), one for each treatment, into the different fields, totaling 20 plots for the whole experiment and five replicate plots per treatment (n = 5). Supplementary Figures 1 and 2 show treatments and sampling times. Removal treatments were implemented in September 2016 and then again in April 2017 due to some resprouting of the

plant invader. The distance between each plot was *c.* 10 m and replicate fields were at least 100 m apart from each other. The annual local rainfall is above 1100 mm and the temperature ranges from 3 to 6 ℃ in winter and from 12 to 15 ℃ in summer (see Gioria and Osborne 2013). On Achill Island, *G. tinctoria* invades alluvial or colluvial soils, derived from volcanic material, or thin gley soils of marine origin with a pH that ranges from 4.6 to 6.2 (Gioria and Osborne 2013).

Maximum litter accumulation and litter quality

To estimate maximum litter accumulation, all surface plant material, such as dead leaves and other herbaceous necromass in uninvaded grasslands, and *G. tinctoria* debris (e.g., leaves and petioles) in invaded areas, was collected in four 0.25 m^2 quadrats, randomly positioned within each plot every month throughout 2017. The study area is dominated by herbaceous species (i.e., grass species in the uninvaded grasslands and invaded sites, which were dominated by *G. tinctoria*; see Mantoani et al. 2020; Mantoani and Osborne 2022), thus woody necromass was not present. We avoided sampling in the same microplots by re-assigning new sampling microplots for each monthly measurement. The material was dried until constant weight at 70 °C and then summed for estimating monthly maximum litter accumulation per m^2 per plot and extrapolated to Mg ha^{-1}. To estimate C:N ratio for plant tissues, for use as a proxy for litter quality, samples from three uninvaded sites and from three *G. tinctoria* plants were harvested throughout 2017 (n = 3), following the harvesting of plants as described in Mantoani et al. (2020). Samples were dried at 70 °C, ground into a fine powder using a mini steel ball grinder, and C and N concentrations were measured by dry combustion in an elemental analyser (Europa Scientific; Iso-Analytical Limited, Crewe, UK).

Earthworm sampling, identification and community diversity

A quantitative hand-sorting method was used for this study. Any decomposing material (i.e., litter) was removed to allow access to the topsoil. For each plot, one soil block (25 × 25 × 30 cm deep) was dug using a metal frame and spade, placed onto a large,

light-coloured plastic sheet (2 m^2) in situ, broken up by hand and sorted carefully. Any earthworms collected were placed into a plastic jar and stored on ice packs, in the dark, in a cooler box. In the laboratory, the animals were rinsed, blotted dry on tissue paper, weighed (biomass data are live biomass with gut content) and preserved in formalin (4%). Then, after preservation for at least 7 days, the samples were transferred into a 70% alcohol solution. A stereoscopic microscope was used for species-level identification of adults, following the taxonomy of Sims and Gerard (1999). Juvenile earthworms were assigned, according to mouth lobe and pigmentation, to one of three groups, namely tanylobic (*Lumbricus* spp.); epilobic dark pigmented (*Aporrectodea longa*); epilobic other (mainly endogeic species).

Earthworm samples were collected four times in total, from 2017 until 2019, during spring and autumn. In north-western Europe, spring and autumn are the peak activity seasons for earthworms and, therefore, the most suitable times for obtaining representative community data (Curry 1994; Sims and Gerard 1999). Given the remoteness of the area of study and the available funding, we were able to collect samples in the spring of 2017, autumn of 2018, spring of 2019, and autumn of 2019.

Statistical analysis

To compare differences in litter accumulation and earthworm abundance and biomass amongst the four treatments, we used general linear mixed-model effects with restricted maximum likelihood, with treatments (i.e., GRASS, GUN, MER, and HER) considered as fixed factors and field considered as random factors. Bonferroni's *post-hoc* correction was applied, and visual analysis of the residuals was also carried out to ensure normality. These analyses were performed with a significance level of $\alpha = 0.05$, using SPSS Statistics v. 24 (IBM 2016).

To calculate species diversity (based on adult earthworms only because juveniles cannot be identified morphologically to species level), each site was treated as a replicate and two measures were used: Species richness (number of species), and the Shannon diversity index, calculated at each site (five replicate sites), for each treatment (four levels: control, Gunnera, herbicidal, and mechanical) and

season (2 levels: spring and autumn). The Shannon diversity index was calculated using the formula:

$$H' = -\sum p_i \ln p_i$$

where p_i is the proportion of individuals found in the ith species (Magurran 2004).

We tested the effect of treatment, season, and their interaction on the diversity of earthworm communities, separately for the two diversity measures, using a two-way ANOVA, with treatment and season as orthogonal factors. Earthworm abundance was expressed as the number of earthworms per m². All analyses were conducted using a significance level α = 0.05. Prior to testing hypotheses concerning mean differences, data were tested for normality and homogeneity of variances using the Cochran's C-test. When interactions were significant, posteriori multiple comparison tests were conducted using the Tukey test.

Multivariate analyses were used to estimate the effect of treatment and season on earthworm communities, separately for adults and juveniles. PERMANOVA (Anderson 2001; McArdle and Anderson 2001) was used to test for the effects of treatment on earthworm communities and its interaction with season (two-way tests). PERMANOVA tests were based on 9999 restricted permutations of the data (α = 0.05). Dissimilarities in species composition were calculated using the Bray-Curtis measure (Bray and Curtis 1957). When significant effects were found, we performed distance-based redundancy analyses (dbRDA) (McArdle and Anderson 2001) to graphically represent the data and predict multivariate variation of earthworm communities in the different treatments in a multivariate space. dbRDA was also based on Bray-Curtis dissimilarities. Differences in the compositional diversity of earthworm communities were visualized using dominance-diversity curves (Whittaker 1965; Clarke and Warwick 2001). For this purpose, species were ranked in decreasing order of importance, based on seed bank abundance. These community analyses were conducted in the R software environment (v. 4.1.0, R Development Core Team 2021). PERMANOVA analyses were performed using the 'PERMANOVA' package (v. 0.1.0; Vicente-Gonzalez and Vicente-Villardon 2021), while the 'vegan' package (v. 2.5–7; Oksanen et al. 2020) was used to perform

distance-based redundancy analysis (*capscale* function).

Results

Litter accumulation and quality

The maximum accumulation of litter varied with sampling time and between treatments ($F_{(11,88)} = 57.19$; $P < 0.001$; Fig. 1). At the end of the growing season, differences between invaded and uninvaded areas were up to 9.94 Mg ha⁻¹ (95% CI = 9.10, 10.78). The maximum litter accumulation was also considerably higher (10.47 ± 0.37 Mg ha⁻¹) in invaded areas compared to uninvaded grasslands (0.54 ± 0.02 Mg ha⁻¹). Based on the lower C:N ratio, *G. tinctoria* produced litter with a higher quality (16.80 ± 0.70) in comparison to uninvaded grasslands (28.65 ± 3.41).

Earthworm abundance and biomass

The abundance of earthworms differed between treatments ($F_{(3,60)} = 9.80$; $P < 0.001$; Fig. 2). Areas invaded by *G. tinctoria* had higher numbers of earthworms per m² in comparison to uninvaded grasslands (95% CI = 124.62, 364.98), mechanically removed plots (95% CI = 5.42, 245.8) and herbicide-treated areas (95% CI = 27.02, 267.38). On average, invasive stands had an almost 3-fold larger earthworm populations (i.e., 375 individuals per m²) than uninvaded areas (i.e., 130 individuals per m²), and 50–64% more earthworms in comparison to areas where plants where mechanically removed (i.e., 250 individuals per m²) or treated with herbicide (i.e., 228 individuals per m²). The highest number of earthworms were found in the spring of 2017, with an average of 300 earthworms per m² across all treatments and *c.* 500 earthworms per m² in areas invaded by *G. tinctoria*.

The biomass of earthworms paralleled their abundance and differences between all four treatments were found ($F_{(3,60)} = 11.24$; $P < 0.001$; Fig. 3). The biomass of earthworm populations in invaded areas was also higher than in uninvaded areas (95% CI = 36.75, 101.78), or areas subjected to mechanical removal (95% CI = 11.39, 76.42) or treated with herbicides (95% CI = 16.64, 81.67). In invaded areas, the biomass of earthworms was approximately 2.5-fold larger (i.e., 114 g per m²) in comparison to uninvaded

Fig. 1 Seasonal variation in litter accumulation (Mg ha^{-1}) throughout 2017, on Achill Island, Co. Mayo, Ireland (n = 5; mean ± 1SE). Legend: GRASS = uninvaded semi-natural grasslands; GUN = areas invaded by *G. tinctoria*. Superscripts show the results of ANOVAs and Tukey tests. The same letters indicate that differences between treatments are not significant.
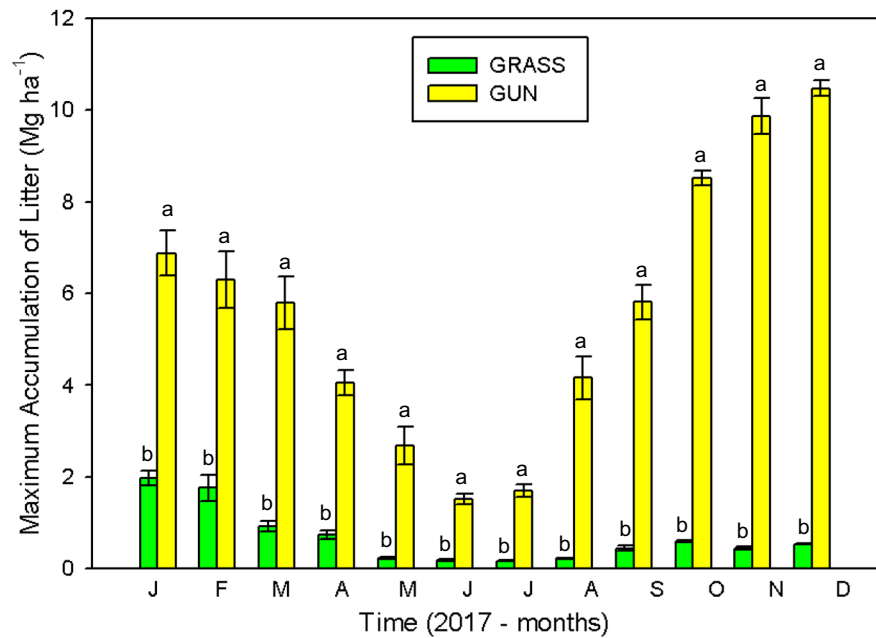

Fig. 2 Abundance of earthworms (individuals per m^2) sampled from 2017 to 2019, on Achill Island, Co. Mayo, Ireland (n = 5; mean ± 1SE). Legend: GRASS = uninvaded semi-natural grasslands; GUN = areas invaded by *G. tinctoria;* MER = mechanical removal; HER = herbicide-treated plots. Superscripts show the results of ANOVAs and Tukey tests. The same letters indicate that differences between treatments are not significant.
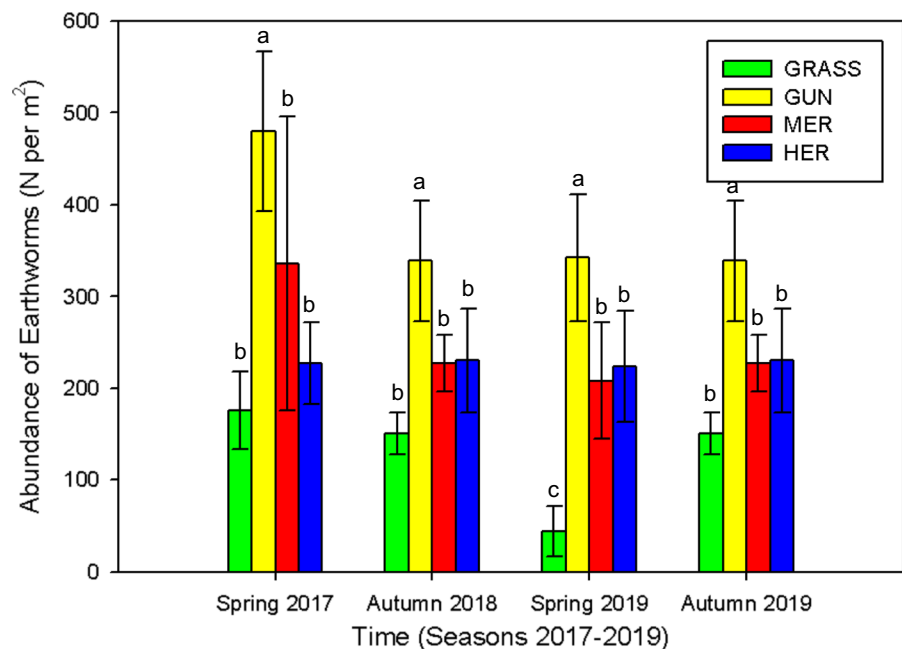

grasslands (i.e., 45 g per m^2), and also substantially larger than in the mechanical removal (i.e., 70 g per m^2) and herbicide (i.e., 65 g per m^2) plots, respectively. Considering seasons, the highest earthworm biomass was found during the autumn, with an average of 83 g per m^2.

Earthworm community diversity

In total, we recorded 10 earthworm species (adults) from the 20 plots across five sites within four treatments (Table 1). The common endogeic species *Allolobophora chlorotica, Aporrectodea caliginosa*

Fig. 3 Biomass of earthworms (2017–2019), on Achill Island, Co. Mayo, Ireland (n = 5; mean ± 1SE). Legend: GRASS = uninvaded semi-natural grasslands; GUN = areas invaded by *G. tinctoria;* MER = mechanical removal; HER = herbicide-treated plots. Superscripts show the results of ANO-VAs and Tukey tests. The same letters indicate that differences between treatments are not significant.
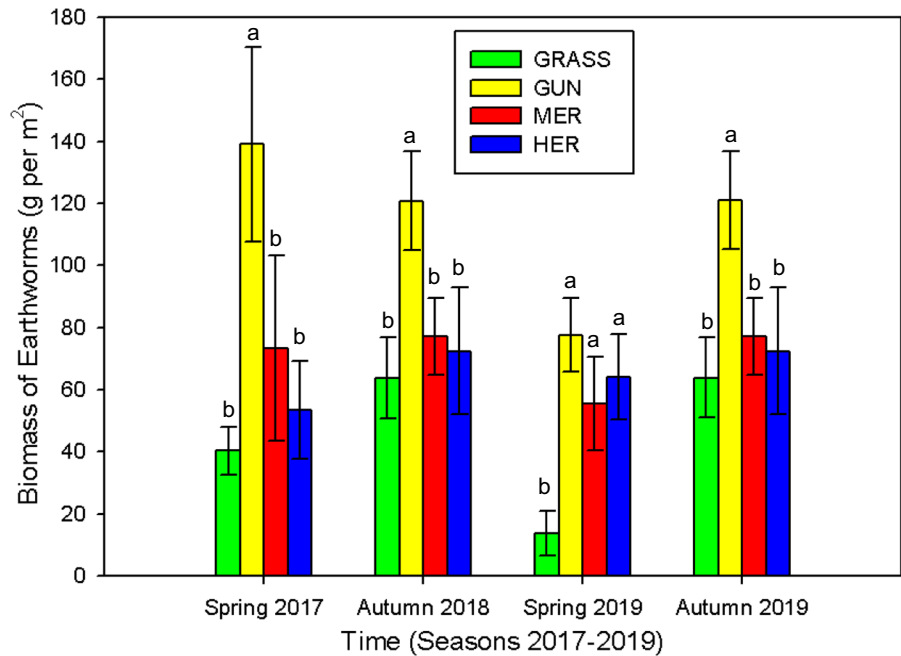
and *Aporrectodea rosea* were the most abundant and most frequent species, while two endogeic *Octolasion* species were also recorded. Among the three epigeic species, only *Lumbricus rubellus* was recorded from all four treatments, but at a relatively low frequency. Two anecic species were recorded, *Aporrectodea longa* and *Lumbricus terrestris,* with low abundances and frequencies for adults. However, juvenile *A. longa*, as well as juvenile *Lumbricus* species, were found in about half of all samples.

The effect of treatment on species richness and Shannon diversity of adult earthworm communities was significant ($P = 0.002$; $P = 0.02$) but that of season was not (Fig. 4). In spring, earthworm communities beneath *G. tinctoria* stands were significantly richer in earthworm species than in uninvaded grasslands ($P_{GUN-GRASS} = 0.001$), or the mechanical removal ($P_{GUN-MER} = 0.01$) and herbicide-treated plots ($P_{GUN-HER} = 0.011$). Similarly, the Shannon diversity index beneath *G. tinctoria* stands was also higher than in any other treatment ($P_{GUN-GRASS} = 0.001$; $P_{GUN-MER} = 0.007$; $P_{GUN-HER} = 0.006$). Differences in species richness or Shannon diversity between treatments were not significant in autumn. PERMANOVA analyses showed a significant effect of treatment on earthworm communities (adults only; $P = 0.017$), while the effect of season on species composition of

adults was not significant. Pairwise tests showed that earthworm communities beneath *G. tinctoria* stands were significantly different from those found in the mechanical removal plots in spring (Pseudo-$P_{GUN-MER} = 0.016$) but not in autumn, as well as from control plots in spring (Pseudo-$P_{GUN-GRASS} = 0.007$). Earthworm communities (adults only) in autumn did not differ significantly between treatments.

The dbRDA vectors indicate that earthworm communities in uninvaded grasslands and invaded plots differed somewhat in species composition (Fig. 5). These differences were driven by the higher abundance of *A. chlorotica*, *A. caliginosa*, and *A. rosea* beneath *G. tinctoria* stands in spring. Further, dominance-diversity curves showed that, in spring, dominance by the three most abundant species (adults) was less pronounced in earthworm communities beneath invasive stands than in any other treatment and that richness was higher beneath *G. tinctoria* (Fig. 6).

For juveniles only, PERMANOVA analyses also showed a significant effect of treatment, which was driven by significant differences in juvenile earthworm communities in uninvaded grasslands and mechanical removal plots in spring (Pseudo-$P_{GRASS-MER} = 0.029$). No significant differences were identified between the other treatments, and no statistically significant differences in juvenile earthworm

Table 1 Abundance, frequency and ecological group abundance of earthworms collected on Achill Island, Co. Mayo, Ireland, during 2017–2019.

Species	Ecological Group	GRASS	GUN	MER	HER
Abundance (individuals per sample)					
Adults					
Allolobophora chlorotica	Endogeic	1.6	4.3	3.2	2.9
Aporrectodea caliginosa	Endogeic	1.8	4.0	3.8	2.6
Aporrectodea longa	Anecic	0.3	0.2	0.1	0.5
Aporrectodea rosea	Endogeic	1.0	2.9	1.7	1.8
Eiseniella tetraedra	Epigeic	0.1	0.6	0.5	0.0
Octolasion cyaneum	Endogeic	0.3	0.2	0.1	0.3
Octolasion tyrtaeum	Endogeic	0.1	0.2	0.1	0.0
Lumbricus castaneus	Epigeic	0.0	0.2	0.1	0.0
Lumbricus rubellus	Epigeic	0.2	1.2	0.2	0.5
Lumbricus terrestris	Anecic	0.0	0.2	0.2	0.0
Juveniles					
Juv. epilobous, unpigmented		2.7	5.3	4.2	4.0
Juv. *A. longa*		0.2	1.4	1.0	1.4
Juv. *Lumbricus* spp.		0.6	2.2	1.1	1.6
Frequency (fraction of all samples with that species)					
Adults					
Allolobophora chlorotica		55	95	75	75
Aporrectodea caliginosa		60	85	85	65
Aporrectodea longa		15	15	5	35
Aporrectodea rosea		45	65	60	55
Eiseniella tetraedra		5	20	5	0
Octolasion cyaneum		25	20	5	20
Octolasion tyrtaeum		5	15	5	0
Lumbricus castaneus		0	15	5	0
Lumbricus rubellus		5	35	15	30
Lumbricus terrestris		0	10	15	0
Juveniles					
Juv. epilobous, unpigmented		70	85	95	75
Juv. *A. longa*		10	40	40	60
Juv. *Lumbricus* spp.		45	60	55	45
Ecological Group Abundance (individuals per sample)					
Endogeic		4.7	11.5	8.7	7.5
Epigeic		0.3	2.0	0.7	0.5
Anecic		0.3	0.3	0.2	0.5

GRASS uninvaded semi-natural grasslands; *GUN* areas invaded by *G. tinctoria; MER* mechanical removal; *HER* herbicide-treated plots.
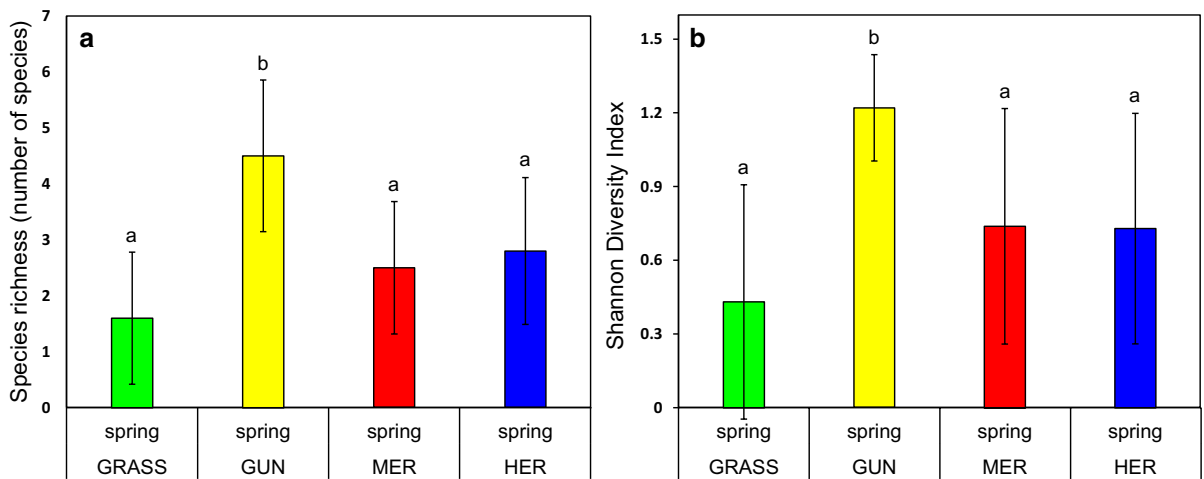
Fig. 4 Mean (±SD) species richness (**a**) and Shannon Diversity Index (**b**) (n = 5 sites) of earthworm communities (adults only) for each of the four treatments (GRASS = uninvaded semi-natural grasslands; GUN = areas invaded by *G. tinctoria;* MER = mechanical removal; HER = herbicide-treated plots.), in spring, over two years. Since differences in autumn were not significant, they are not represented here. Superscripts show the results of ANOVAs and Tukey tests. The same letters indicate that differences between treatments are not significant.

Fig. 5 Distance based redundancy analysis (dbRDA) describing patterns in earthworm communities (adults only) in spring at five sites (replicates) for each of four treatments (GRASS = uninvaded semi-natural grasslands; GUN = areas invaded by *G. tinctoria;* MER = mechanical removal; HER = herbicide-treated plots). Each point corresponds to the earthworm communities at each site, for each treatment.
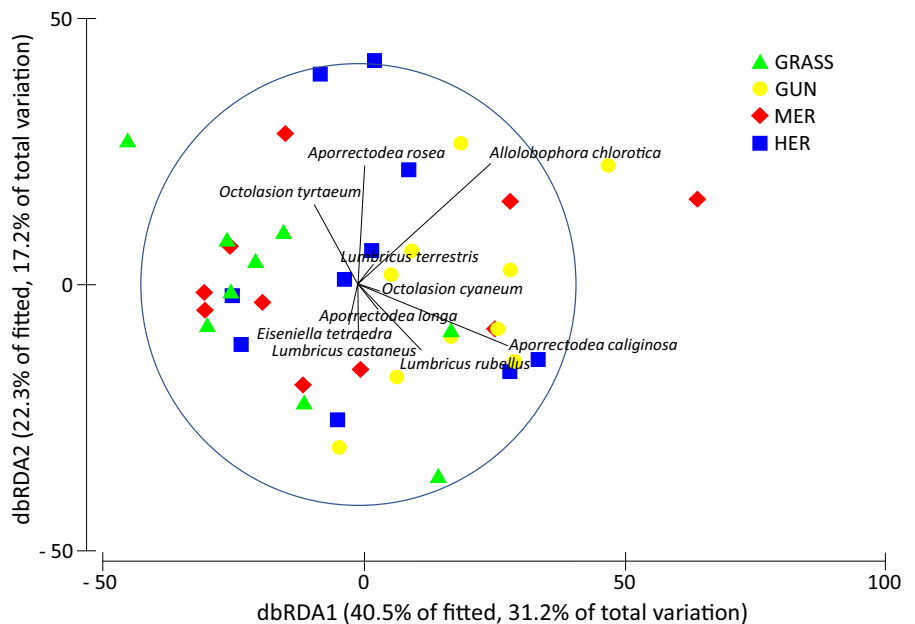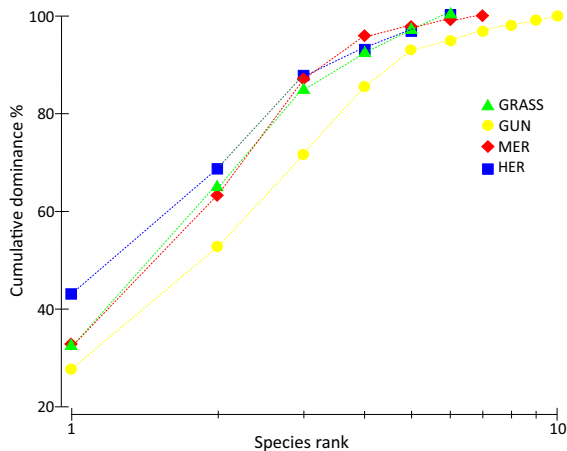
Fig. 6 Dominance-diversity curves based on earthworm data (adults only) collected at five sites (replicates) for each of four treatments (GRASS = uninvaded semi-natural grasslands; GUN = areas invaded by *G. tinctoria;* MER = mechanical removal; HER = herbicide-treated plots). Each point represents the average abundance of individual species at five sites, ranked based on their relative abundance, within each treatment.

communities were observed in autumn (data not shown).

Discussion

Litter accumulation and litter quality

Increased litter (Ehrenfeld 2003; Wolkovich et al. 2010; Tamura and Tharayil 2014) and a higher biomass production (Sakai et al. 2001; Bossdorf et al. 2005) are features associated with many invasive plant species. Consistent with this and earlier studies (Gioria et al. 2011; Gioria and Osborne 2013; Mantoani et al. 2020), *G. tinctoria* invasions in the present study were also associated with significantly larger litter inputs, with *c.* 20-fold more litter accumulation than comparable uninvaded grasslands. In addition to the increased availability of litter associated with invasive stands, the much lower C:N ratios (17 vs. 29) indicated that the quality of *G. tinctoria* litter was also likely higher than that from the uninvaded grasslands. However, the palatability of litter to earthworms may also depend on the phenolic content of plant tissues (Hendriksen 1990), which is notably higher in *G. tinctoria* (Zamorano et al. 2017). Hendriksen (1990)

indicated, for instance, that the palatability of litter (from deciduous tree species) to earthworms may depend more on the phenolic content rather than the C:N ratio alone. Microbial colonisation of decomposing litter is also known to influence earthworm feeding (Curry and Schmidt 2007). For example, in experiments with the anecic *L. terrestris*, Schönholzer et al. (1998) observed a decrease in bacterial biomass and palatability of *Hypericum perforatum* (St. John's wort) leaf litter and ascribed that to the presence of polyphenolic secondary compounds.

Earthworm abundance, biomass and diversity

Contrary to our original hypothesis, areas invaded by *G. tinctoria* had a 2 to 3-fold larger earthworm abundance and biomass compared to uninvaded grasslands. Even where *G. tinctoria* plants had been removed, 50--64% more earthworms were present than under grass for over two-years after the implementation of the treatments, demonstrating a significant soil legacy effect of the invasions on earthworm abundance. Remarkably, the overall earthworm abundances and biomasses observed here in seminatural grassland (130 individuals m^{-2}, 45 g biomass m^{-2}) and under *G. tinctoria* (375 individuals m^{-2}, 114 g biomass m^{-2}) were very similar to those estimated (also with the hand-sorting method) in an Irish national survey that included 8 'rough grazing' sites and 21 'improved pasture' sites, respectively (Keith et al. 2012). In other words, *G. tinctoria* invasions had the same beneficial effect on earthworm populations as the fertilization, liming and associated plant productivity increase associated with 'improved pasture' systems. This finding contradicts the result of a recent meta-analysis (Abgrall et al. 2019) suggesting that soil detritivores are reduced by invasive plants in 'open habitats' such as grasslands but increased in 'closed habitats' such as forests (6 and 14 studies, respectively).

Clearly, there was a link between the increased amount of litter associated with invasive stands and earthworm abundance/biomass, with an approximately 20-fold increase in (above-ground) litter associated with a two-to-three-fold increase in earthworm population sizes. However, aggregated data on earthworm ecological groups (Table 1) suggest that there was no direct trophic benefit from the increased *G. tinctoria* litter for the large-bodied,

anecic group that feeds on surface litter. On the other hand, the epigeic species increased substantially, even though that increase was less marked than in an unploughed system with a permanent white clover cover, which is presumed to provide a highly nutritious litter (e.g., Schmidt et al. 2003). These findings suggest that the palatability of *G. tinctoria* litter for litter-feeding earthworms is controlled by an interplay of the (favourable) C:N ratio measured here and the (potentially unfavourable) high concentration of phenolics known from studies of *G. tinctoria* as a medicinal plant (Zamorano et al. 2017; Hebel-Gerber et al. 2021).

Regarding the total phenolic content of *G. tinctoria*, Zamorano et al. (2017) have demonstrated that leaves had at least 10 times more phenolic compounds than in common vegetables, and Hebel-Gerber et al. (2021) have shown that petioles contain many antimicrobial molecules, which, in turn, may reduce microbial colonisation of *G. tinctoria* litter. Overall, the C:N ratio seems to be a more important factor determining the palatability of litter to earthworms, in contrast to the findings of Hendriksen (1990). However, it is possible that the impact of high phenolics on earthworm feeding may have been over-emphasized in the past given the demonstration that they contain unique metabolites in their guts that counter the inhibitory effects of polyphenols (Liebeke et al. 2015). Moreover, whilst it is known that seeds and seedlings can be a significant source of nutrition for soil earthworms (Eisenhauer et al. 2010), it is unclear to what extent the larger seed bank associated with *G. tinctoria* invasions (Gioria and Osborne 2009; 2010; Gioria et al. 2011) support a larger earthworm population.

Environmental differences between invaded and uninvaded areas may also be important. For instance, the higher soil pH in invaded areas (7.3 in invaded areas in comparison to 6.2 in uninvaded grasslands; Mantoani 2019) could have contributed to an increase in earthworm populations, in line with several grassland studies (reviewed by Syers and Springett, 1983). However, Cray et al. (2021) did not observe an increase in earthworm abundance in Canadian tallgrass prairies when the soil pH was increased from 5.3 to 7.8. Interestingly, the higher soil temperatures in the uninvaded grasslands during May-August (reported for 2016 and 2017 in Mantoani et al. 2020) may be a contributing factor restricting earthworm activity as well. Even though bulk soil moisture

contents were not significantly different between invaded and uninvaded areas (Mantoani 2019), the higher abundance of the semi-aquatic species *Eiseniella tetraedra* in *G. tinctoria* stands (Table 1) suggests that there may have been more suitable micro-habitats in invasive stands where this species' requirement for high moisture was better met than in the uninvaded fields. Overall, all the earthworm communities observed in this study were typical of Irish grasslands (Keith et al. 2012; Bacher et al. 2018) and *G. tinctoria* invasions did not result in any unusual changes in community features.

Although it might be expected that enhanced litter production would favour epigeic earthworms that live on or close to the soil surface, the abundance of the soil-feeding, endogeic species roughly doubled as well, and they continued to be dominant in the earthworm community under *G. tinctoria*. We know from radiocarbon studies that the epigeic *L. rubellus* assimilates recently fixed C (0–3 years old), while the endogeic *A. caliginosa* assimilates older C (5–8 years old) (Briones et al. 2005). The present results suggest that the products (and microbes) associated with the decomposition of *G. tinctoria* litter in these old stands may pass the digested products on to the endogeic functional group that, in turn, benefits from this processed food supply. There is now also a growing consensus (at least for forest systems) that below-ground C inputs are an important trophic route for soil decomposer animals including endogeic earthworms (Pollierer et al. 2007). However, this is less certain for herbaceous plant species. Interestingly, root biomass is significantly reduced in areas invaded by *G. tinctoria* (Mantoani and Osborne 2021), making it less likely that the below-ground route could explain the larger earthworm populations.

The plots where *G. tinctoria* plants were either physically removed or chemically controlled had earthworm population sizes that were intermediate between invaded and uninvaded areas (Figs. 2 and 3). The finding that higher earthworm abundance and biomass was maintained for approximately two years after the implementation of the removal treatments suggests that some earthworms persisted and continue to benefit for some time on partially decomposed *G. tinctoria* litter. However, contrary to our second hypothesis, this legacy effect was beneficial rather than detrimental. It also needs to be remembered that the observed population changes in the

treatment plots (5 ×5 m), located inside *G. tinctoria* stands, may reflect earthworm mobility and microhabitat choice, rather than true resident population changes, because earthworms have a mobility that is relevant to that scale (Mather and Christensen 1988). For instance, in grassland experiments in Ireland, Bacher et al. (2018) showed that most species are mobile (in this case, they moved towards dung pats), and they estimated that the maximum size of the recruitment area from which earthworms moved to dung pats was 3.8 m^2. In the present study, it is therefore possible that some earthworms moved out of the removal plots, into *G. tinctoria* stands, which would imply that any beneficial legacy effect could last longer in larger areas where earthworms persist.

Implications

It is predicted that invasive species may have different effects depending on the trophic level, with a greater impact linked to the lower trophic level from the invader (Bradley et al. 2016; McCary et al. 2016), although direct evidence for this is still limited. However, this is almost always considered in a negative context (i.e., detrimental impact; Schirmel et al. 2016), whereas the results of the present work show a major beneficial effect of *G. tinctoria* invasions on native earthworm populations, not a detrimental one. As a higher litter production is often a feature of plant invaders, a general increase in earthworm populations, might, consequently, be a common response to alien plant introductions. Nevertheless, this may be critically dependent on the C:N ratio of the litter. As *G. tinctoria* is an N-fixing invasive species (Osborne et al. 1991; Mantoani et al. 2020) it will typically have a low tissue C:N ratio, whilst most plant invaders are not N-fixing and likely to have a higher tissue C:N ratio and a less palatable litter. However, there is little information on the effect of plant invasions on soil animals, including earthworm populations (Abgrall et al. 2019) and further work is required to assess the generality of the findings of the current study. Another important point that warrants research is the potentially contrasting impacts of plant invaders on different communities with beneficial impacts on some communities (i.e., earthworms) as in the current study, while having detrimental effects on others (i.e., plants and other animals). This indicates that a more holistic approach that examines the impacts of invasive plants on different trophic-levels and ecosystem processes is required to assess the justification, as well as the feasibility of management/eradication and restoration measures.

Declarations

References

Abgrall C, Forey E, Chauvat M (2019) Soil fauna responses to invasive alien plants are determined by trophic groups and habitat structure: a global meta-analysis. Oikos 128:1390–1401

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Bacher MG, Fenton O, Bondi G, Creamer RE, Karmarkar M, Schmidt O (2018) The impact of cattle dung pats on earthworm distribution in grazed pastures. BMC Ecol 18:59

Belnap J, Phillips SL (2001) Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecol Appl 11:1261–1275

Blouin M, Hodson ME, Delgado EA, Baker G, Brussaard L, Butt KR, Dai J, Dendooven L, Peres G, Tondoh JE, Cluzeau D, Brun JJ (2013) A review of earthworm impact on soil function and ecosystem services. Eur J Soil Sci 64:161–182

Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11

Bouché MB (1972) Lombriciens de France: Écologie et Systématique. Institut National de la Recherche Agronomique, Paris, p 671

Bradley BA, Laginhas BB, Whitlock R, Allen JM, Bates AE, Bernatchez G, Diez JM, Early R, Lenoir J, Vila M, Sorte CJB (2016) Disentangling the abundance-impact relationship for invasive species. Proc Nat Acad Sci 116:9919–9924

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of Southern Wisconsin. Ecol Monogr 27:325–349

Briones MJI, Garnett MH, Piearce TG (2005) Earthworm ecological groupings based on ^{14}C analysis. Soil Biol Biochem 37:2145–2149

Callaway RM, Thelen GC, Rodriguez A, Holben D (2004) Soil biota and exotic plant invasion. Nature 427:731–733

Clarke K, Warwick R (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth, UK

Cray HA, Gaudon JM, Murphy SD (2021) Introduced earthworms (Lumbricidae) in restored and remnant tallgrass prairies of southern Ontario. Can Field Nat 135:234–244

Curry JP (1994) Grassland invertebrates: Ecology, influence on soil fertility and effects on plant growth. Chapman & Hall, London

Curry JP, Schmidt O (2007) The feeding ecology of earthworms – a review. Pedobiologia 50:463–477

D'Antonio C, Flory SL (2017) Long-term dynamics and impacts of plant invasions. J Ecol 105:1459–1461

Diagne C, Leroy B, Vaissiere A-C, Gozlan RE, Roiz D, Jaric I, Salles J-M, Bradshaw CJA, Corchamp F (2021) High and rising economic costs of biological invasions worldwide. Nature 592:571–576

Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–523

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst 41:59–80

Eisenhauer N, Butenschoen O, Radsick S, Scheu S (2010) Earthworms as seedling predators: importance of seeds and seedlings for earthworm nutrition. Soil Biol Biochem 42:1245–1252

Fennell M, Gallagher T, Vintro LL, Osborne BA (2014) Using soil seed banks to assess temporal patterns of genetic variation in invasive plant populations. Ecol Evol 4:1648–1658

Gaudon JM, McTavish MJ, Sivarajah S, Snyder EHU, Andrade SMM, Murphy SD (2020) Effects of earthworms and warming on tree seedling growth: a small-scale microcosm experiment. Can J For Res 50(11):1253–1257

Gioria M, Osborne BA (2009) The impact of *Gunnera tinctoria* (Molina) Mirbel invasions on soil seed bank communities. J Plant Ecol 2:153–167

Gioria M, Osborne BA (2010) Similarities in the impact of three large invasive plant species on soil seed bank communities. Biol Invasions 12:1671–168

Gioria M, Osborne B (2013) Biological flora of the British Isles: *Gunnera tinctoria*. J Ecol 101:243–264

Gioria M, Dieterich B, Osborne B (2011) Battle of the giants: primary and secondary invasions by large herbaceous species. Biol Environ: Proc R Ir Acad 111B:177–193

Guerin GR, Martín-Forés I, Sparrow B, Lowe AJ (2017) The biodiversity impacts of non-native species should not be extrapolated from biased single-species studies. Biodivers Conserv. 27:1–6

Hebel-Gerber S, García-Cancino A, Urbina A, Simirgiotis MJ, Echeverría J, Bustamante-Salazar L, Sáez-Carrillo K, Alarcón J, Pastene-Navarrete E (2021) Chilean Rhubarb, Gunnera tinctoria (Molina) Mirb. (Gunneraceae): UHPLC-ESI-Orbitrap-MS profiling of aqueous extract and its anti-helicobacter pylori activity. Front Pharmacol 11:583961

Hendriksen NB (1990) Leaf litter selection by detritivore and geophagous earthworms. Biol Fertil Soils 10:17–21

IBM Corp (2016) IBM ®SPSS ®Statistics for Windows, Version 24; IBM Corp: Armonk, NY, USA

Keith AM, Boots B, Hazard C, Niechoj R, Arroyo J, Bending GD, Bolger T, Breen J, Clipson N, Doohan FM, Griffin CT, Schmidt O (2012) Cross-taxa congruence, indicators and environmental drivers in soils under agricultural and extensive land management. Eur J Soil Biol 49:55–62

Kourtev PS, Ehrenfeld JG, Haggblom MH (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biol Biochem 35:895–905

Kuebbing SE, Nuñez MA (2018) Current understanding of invasive species impacts cannot be ignored: potential publication biases do not invalidate findings. Biodivers Conserv 106:687–698

Le Bayon RC, Bullinger-Weber G, Schomburg AC, Turberg P, Schlaepfer R, Guenat C (2017) Earthworms as ecosystem engineers: a review. In: In Horton GC (ed) Earthworms. Types roles and research. Nova Science Publishers, New York, pp 129–177

Liebeke M, Strittmatter N, Fearn S, Morgan AJ, Kille P, Fuchser J et al (2015) Unique metabolites protect earthworms against plant polyphenols. Nat Commun 6:7869

Magurran AE (2004) Measuring biological diversity. Blackwell Publishing, Hoboken NJ

Mantoani MC, Osborne BA (2021) Alien plant introductions and greenhouse gas emissions: insights from *Gunnera tinctoria* invasions. Sci Total Environ 775:145861

Mantoani MC, Osborne BA (2022) Post-invasion recovery of plant communities colonised by *Gunnera tinctoria* after mechanical removal or herbicide application and its interaction with an extreme weather event. Plants 11:1224

Mantoani MC, González AB, Sancho LG, Osborne BA (2020) Growth, phenology and N-utilization by invasive populations of *Gunnera tinctoria*. J Plant Ecol 13:589–600

Mantoani MC (2019) *Gunnera tinctoria* Invasions: phenology, ecosystem impacts and environmental interactions. (PhD thesis). University College Dublin, Ireland

Mather JG, Christensen O (1988) Surface movements of earthworms in agricultural land. Pedobiologia 32:399–405

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analyses. Ecology 82:290–297

McCary M, Mores RM, Farfan MA, Wise D (2016) Invasive plants have different effects on trophic structure of green and brown food webs in terrestrial ecosystems: a meta-analysis. Ecol Lett 19:328–335

Meisner A, Hol WHG, de Boer W, Krumins JA, Wardle DA, van der Puten WH (2014) Plant-soil feedbacks of exotic species across life forms: a meta-analysis. Biol Invasions 16:2551–2561

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, et al. (2020) Community ecology package. https://cran.r-project.org/web/packages/vegan/vegan.pdf

Osborne BA, Doris F, Cullen A, McDonald R, Campbell G, Steer M (1991) *Gunnera tinctoria*: an unusual nitrogen-fixing invader. BioScience 41:224–234

Pollierer MM, Langel R, Körner C, Maraun M, Scheu S (2007) The underestimated importance of belowground carbon input for forest soil animal food webs. Ecol Lett 10:729–736

R Development Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org.

Ricardi A, Blackburn TM, Carlton JT, Dick JTA, Hulme PE, Lacarella JC, Jeschke JM, Liebhold AM, Lockwood JL, MacIsaac HJ, Pysek P, Richardson DM, Ruiz GM, Simberloff D, Sutherland WJ, Wardle DA, Aldridge DC (2017) Invasion Science: a horizon scan of emerging challenges and opportunities. Trends Ecol Evolution 32:464–474

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC et al (2001) The population biology of invasive species. Ann Rev Ecol Syst 32:305–332

Sapsford SJ, Brandt AJ, Davis KT, Peralta G, Dickie IA, Gibson RD, Green JL, Hulme PE, Nuñez MA, Orwin KH, Pauchard A, Wardle DA, Peltzer DA (2020) Towards a framework for understanding the context dependence of impacts of non-native tree species. Funct Ecol 34:944–955

Schirmel J, Bundschuh M, Entling MH, Kowarik I, Buchholz S (2016) Impacts of invasive plants on resident animals across ecosystems, taxa, and feeding types: a global assessment. Glob Change Biol 22:594–603

Schmidt O, Clements RO, Donaldson G (2003) Why do cereal–legume intercrops support large earthworm populations? Appl Soil Ecol 22:181–190

Schönholzer F, Kohli L, Hahn D, Daniel O, Goez C, Zeyer J (1998) Effects of decomposition of leaves on bacterial biomass and on palatability to *Lumbricus terrestris* L. Soil Biol Biochem 30:1805–1813

Simberloff D, Von Holle B (1999) Positive interactions of non-indigenous species: invasional meltdown? Biol Invasions 1:21–32

Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, Garcia Berthous E, Pascale M, Pysek P, Sousa R, Tabacchi E, Vila M (2013) Impacts of biological invasions: what's what and the way forward. Trends Ecol Evolution 28:58–66

Sims RW, Gerard BM (1999) Earthworms: notes for the identification of British species. Field Studies Council, Shrewsbury

Singh S, Singh J, Vig AP (2016) Effect of abiotic factors on the distribution of earthworms in different land use patterns. J Basic Appl Zool 74:41–50

Syers JK, Springett JA (1983) Earthworm ecology in grassland soils. In: Satchell JE (ed) Earthworm Ecology. Springer, Dordrecht, pp 67–83

Tamura M, Tharayil N (2014) Plant litter chemistry and microbial priming regulate the accrual, composition and stability of soil carbon in invaded ecosystems. New Phytol 203:110–124

Vicente-Gonzalez L, Vicente-Villardon JL (2021) Multivariate analysis of variance based on distances and permutations. https://cran.r-project.org/web/packages/PERMANOVA/PERMANOVA.pdf

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarosik V, Maron JL, Pergl J, Schaffner U, Sun Y, Pysek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities, and ecosystems. Ecol Lett 14:702–708

Vimercati G, Kumschick S, Probert AF, Volery L, Bacher S (2020) The importance of assessing positive and beneficial impacts of alien species. NeoBiota 62:525–545

Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrook R (1997) Introduced species: a significant component of human-caused global change. N Z J Ecol 21:1–16

Whittaker RH (1965) Dominance and diversity in land plant communities. Science 147:250–260

Wolkovich EM, Lipson DA, Virginia RA, Cottingham KL, Bolger DT (2010) Grass invasion causes rapid increases in ecosystem carbon and nitrogen storage in a semiarid shrubland. Glob Change Biol 16:1351–1365

Zamorano P, Rojano B, Morales M, Magarinos H, Godoy P, Munoz O (2017) Biological and antioxidant activity of *Gunnera tinctoria* (Nalca). J Med Plants Res 11:318–330