

This is a “preproof” accepted article for Invasive Plant Science and Management.

This version may be subject to change in the production process, *and does not include access to supplementary material*. DOI: 10.1017/inp.2025.12

## ***Gunnera* soil detrital food web**

### **Changes in the soil detrital food chain associated with *Gunnera tinctoria* plant invasions**

Maurício Cruz Mantoani<sup>1,2</sup>, Olaf Schmidt<sup>3,4</sup>, Sean Storey<sup>1</sup>, Evelyn Doyle<sup>1,3</sup>, Luis Leon Vintro<sup>5</sup>,  
Bruce Arthur Osborne<sup>1,3,4\*</sup>

<sup>1</sup>UCD School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland. (ORCID: 0000-0002-1514-6200)

<sup>2</sup>Institute of Astronomy, Geophysics and Atmospheric Science, University of São Paulo, São Paulo, Brazil

<sup>3</sup>UCD Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland.

<sup>4</sup>UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland.

<sup>5</sup>UCD School of Physics, University College Dublin, Belfield, Dublin 4, Ireland.

**Author for correspondence:** \*bruce.osborne@ucd.ie; +35317167076

This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is unaltered and is properly cited. The written permission of Cambridge University Press must be obtained for commercial re-use or in order to create a derivative work.

**Abstract:** Whilst invasive plants can modify community and ecosystem attributes, there is little information on their impact across different trophic levels. Here, we examined the effects of *Gunnera tinctoria* Molina (Mirb.; *Gunneraceae*; “Chilean-rhubarb”) on the soil detrital food chain, the major pathway for energy flow in terrestrial ecosystems and the recycling of nutrients. Measurements of soil physio-chemistry properties and the natural abundances of C and N isotopes, along with assessments of microbial and earthworm populations were used to verify if *G. tinctoria* drives the soil food web and how C is transferred from decomposing litter to worm populations. Based on the C isotope compositions of plant material and detritivorous worms (i.e., earthworms and enchytraeid worms), all worms contribute to *G. tinctoria* litter decomposition, irrespective of their feeding preference. The increased role of larger earthworm populations in litter decomposition was also associated with changes in the soil microbial community, with some bacterial genera being absent from invaded areas. Of particular interest was a three-fold increase in *Nitrospira* spp. in invaded areas, likely due to an increase in earthworm-related ammonia production, with this being rapidly converted to nitrate. Given the poor representation of other ammonia and nitrite oxidizing soil bacteria, many of the *Nitrospira* spp. present in invaded soils may perform complete ammonia oxidation (i.e., COMAMMOX). Our results indicate a greater role for native earthworms in decomposition processes in areas invaded by *G. tinctoria*, resulting in modifications in the other soil biota and an increase in soil nitrate.

**Keywords:** earthworms; *Nitrospira*; grasslands; invasive plants; isotopes; soil bacteria.

## Management Implications:

The development of advanced management interventions for dealing with invasive plants requires a more holistic approach that recognises the multi-faceted effects that such invasion processes might have, including potentially negative, neutral or positive impacts, both above- and below-ground. To date, however, such evidence is scarce and often focused solely on above-ground effects and changes in the resident plant community with little information on soil organisms or interactions between different trophic levels that are involved in decomposition processes. This is particularly important since a common feature of many plant introductions is a dramatically enhanced and often qualitatively modified litter production whose decomposition will likely have knock-on-effects on soils and soil organisms, as well as on the drivers of soil biogeochemical processes. In this study, we show that *G. tinctoria* invasions can result in a shift in the soil biota and modifications in the detrital food chain, with an increased role for earthworms in decomposition processes and changes in the soil microbial population. This was linked to a 20-fold higher and qualitatively superior (i.e., lower C:N ratio) litter production compared to that found in uninvaded grasslands. These, largely below-ground effects, driven mainly by the increased inputs of higher quality litter, may compromise the effectiveness of any measures aimed at restoring the original plant community. Thus, the removal of plants might lead to carbon loss and a reduction in earthworm and enchytraeid populations. Our results extend the understanding of the complexity of impacts that an invasive species may have on ecosystems and their processes, highlighting the need for more work that encompasses a wide range of invasive species to assess the generality of these results and that tackles multiple aspects of invasion processes at the same time.

## Introduction

Invasive plants are known to have a range of impacts on ecosystems and the services they provide, although much of the focus has been on their effects on the standing vegetation and resident animals (Vilà *et al.* 2011), while much less is known about below-ground effects (McCary *et al.* 2016). Interestingly, the effects on the above-ground vegetation/animals are overwhelmingly negative (Vilà *et al.* 2011), whilst the impacts below-ground appear to be small, insignificant or variable (McCary *et al.* 2016; Pearson *et al.* 2024). Although documented changes in soil chemical properties (Ehrenfeld 2003; Ehrenfeld 2010) and shifts in plant communities and their composition (Vilà *et al.* 2011) might impact on the soil fauna (Zhang *et al.* 2019) and soil bacterial communities (Gibbons *et al.* 2017; McTee *et al.* 2017), few studies have specifically focused on below-ground impacts and their effects on distinct trophic levels at the same time.

Of particular importance, as far as energy flow and nutrient cycling is concerned, is the detrital (decomposer) food chain. This is a predominantly soil/below-ground process that is involved in the recycling of nutrients and represents the major energy flow within a terrestrial ecosystem. This is driven largely by litter inputs and the activity of soil organisms, and since plant invasions are typically associated with high litter inputs (Wolkovich *et al.* 2010), this pathway may be of even greater significance for plant invaders. The extent to which invasive plants lead to ecosystem impacts via nutrient enrichment and associated modifications in soil biogeochemistry and its biota, may depend, therefore, on the extent of decomposition of the generally larger litter inputs. Around 90% of nutrient cycling is thought to be associated with decomposition processes (Mary *et al.* 1996). For many highly productive plant invaders, such as *Gunnera tinctoria*, that have few natural enemies, grazing losses are likely to be less significant, placing an even greater emphasis on the detrital food chain.

Although the focus of many decomposition studies has been on the role of microorganisms, such as bacteria and fungi, the soil fauna can also play an important role (Griffiths *et al.* 2021). There is an increased recognition, for instance, of the role of earthworms in primary decomposition processes (Curry & Schmidt 2007). Recent evidence also suggests that earthworms can rapidly transfer nutrients to plants and are not solely restricted to slow decomposition processes (Shutenko *et al.* 2022). Importantly, there is also evidence that

earthworms can drive the composition of soil bacteria communities through their contribution to the early stages of organic matter degradation (Medina-Sauza *et al.* 2019). Their much smaller relatives, enchytraeid or pot worms (family *Enchytraeidae*) are studied less often but are likely similarly important (Didden 1993); they were therefore also included in the present study.

*Gunnera tinctoria* Molina (Mirb.), commonly known as ‘Chilean-rhubarb’, is a South American species which has become invasive in Ireland, in the UK, in France, in Portugal (Azores Archipelago), in New Zealand and in the USA (Gioria & Osborne 2013). This species forms monospecific stands and invades a wide range of habitats including cliffs, ditches and stream banks in environments typically characterised by high rainfall and moderate temperatures (Gioria & Osborne 2013), impacting particularly on grasslands in Ireland due to its huge biomass productivity (Mantoani *et al.* 2020). *G. tinctoria* belongs to the family *Gunneraceae*, the only angiosperm genus known to establish an endosymbiotic relationship with the cyanobacterium *Nostoc punctiforme* (Osborne & Bergman 2009; Santi *et al.* 2013), which gives it the ability to obtain N from the atmosphere via N-fixation (Osborne *et al.* 1991; Osborne & Sprent 2002) and may be partially responsible for its success as a plant invader (Mantoani *et al.* 2020). This invasive plant species excludes native plants due largely to the development of an extensive canopy that shades out co-occurring species (Mantoani *et al.* 2020), alters the local soil greenhouse gas balance (Mantoani & Osborne 2021a), potentially enhancing N<sub>2</sub>O emissions through facilitated mechanisms (Mantoani & Osborne 2021b) and can survive extreme weather conditions, enabling it to recolonize areas quickly (Mantoani & Osborne 2022a; Mantoani *et al.*, 2025).

In a recent paper we showed that invasions by *G. tinctoria* can result in a marked increase in the abundance and diversity of native earthworms, alongside a 20-fold annual increase in plant litter (Mantoani *et al.* 2022). However, earthworms can also feed on roots, seeds, fungi, and soil bacteria (Curry & Schmidt 2007). To confirm and expand these results, we now provide evidence using stable isotope ratio measurements. For this, we tested the following hypotheses: (1) earthworms and enchytraeids feed directly on the *G. tinctoria* litter, which can be detected by stable isotope ratio measurements; (2) earthworm populations have the capacity to consume most of the litter produced by the plant invader (Curry & Schmidt 2007); and (3) earthworm- and enchytraeid-related changes in the detrital food chain have knock-on-effects on the soil microbial communities, as well as on N cycling (Jang *et al.* 2022).

## Material and Methods

### Area of Study and Experimental Design

The study area is the same as that reported in Mantoani *et al.* (2020; 2022) and Mantoani and Osborne (2021; 2022), and was located in Dooega Beach, Achill Island (53°51' and 54°01' N; and 9°55' and 10°15' W), Co. Mayo, on the west coast of Ireland. Five replicate-fields containing two plots of 5 x 5 m each (25 m<sup>2</sup>), one in uninvaded semi-natural grasslands (GRASS) and another one in areas invaded by *G. tinctoria* (GUN) were used in the study (n = 5 for each treatment). The distance between treatment plots was *ca.* 10 m and each replicate was at least 100 m apart from each other. Invaded plots were characterised by having a high coverage of *G. tinctoria* (i.e., 100% in summer) and low regeneration of native species (*ca.* 10 species or lower), only being colonised by *Agrostis capillaris*, *A. stolonifera*, *Equisetum arvense*, *E. fluviatile*, *Epilobium obscurum*, *Galium aparine*, *Juncus effusus*, *Ranunculus acris*, *R. repens*, and *Urtica dioica*, whereas uninvaded ones had 27–32 species that were absent in invasive stands (Mantoani & Osborne 2022; Gioria *et al.* 2023). In addition to the species mentioned previously, uninvaded grasslands were characterised by native grasses such as *Alopecurus pratensis*, *Festuca ovina*, *Holcus spp.*, *Lolium perenne*, *Poa spp.*, as well as common Irish grasslands species, for example, *Angelica sylvestris*, *Apium nodiflorum*, *Cirsium palustre* and *C. vulgare*, *Lythrum salicaria*, *Mentha aquatica*, *Prunella vulgaris*, *Rubus fruticosus*, *Rumex spp.*, *Trifolium pratense* and *T. repens*. Local annual rainfall is above 1,100 mm and the temperature ranges from 3 to 6 °C in winter and from 12 to 15 °C in summer (Gioria & Osborne 2013). On Achill Island, *G. tinctoria* invades alluvial or colluvial soils, derived from thin gley material of marine origin or from volcanic material, with a pH between 4.6 to 6.2 (Gioria & Osborne 2013).

### Earthworm Sampling, Plant Harvesting, and Isotope Ratio Analyses

Following the sampling protocol described in Mantoani *et al.* (2022), earthworms (family *Lumbricidae*) and enchytraeid worms (family *Enchytraeidae*) (both subclass Oligochaeta) were collected in June 2017 for isotopic analyses. In brief, soil blocks (25 x 25 x 30 cm) were hand-sorted and adults of *Allolobophora chlorotica* (a small, endogeic, soil-feeding

species) and of *Lumbricus rubellus* (a medium-sized, epigeic, litter-feeding species), as well as enchytraeid worms that were visible under field conditions were collected manually. Both earthworm species are native to Ireland; enchytraeid species were not determined. All worms were kept live in a cooler box, transported to the laboratory, and allowed to void their guts on moist paper tissue for 24 hours. Live plant material was also collected from both treatments, and separated into leaf, petiole, and rhizome for *G. tinctoria*, and leaves and roots for grassland plants.

Soil samples and rinsed plant materials were oven-dried (50°C) and powdered using a ball mill, while earthworms were freeze-dried and crushed individually using a mortar and pestle. Subsamples of about 1 mg dry weight (earthworms) and 4 mg dry weight (soil and plants) were weighed into miniature tin capsules (4 by 6 mm) and sealed. Several freeze-dried enchytraeid worms were pooled to achieve about 1 mg dry weight per cup. Samples were analysed by elemental analysis – continuous flow isotope ratio mass spectrometry (EA-CF-IRMS), in dual isotope mode, using a Europa Scientific EA and Europa Scientific 20-20 IRMS instrument. Isotope ratios ( $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ) are expressed in conventional delta ( $\delta$ ) notation in parts per thousand (‰). Analytical precision (standard deviation,  $n=8$ ) of check samples (wheat flour) analysed along with the samples was 0.01‰ and 0.08‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. More details on the isotopic analysis are reported in Mantoani *et al.* (2020).

### **Soil Sampling – Soil Profile and Bulk Density**

Soil samples were taken from three soil depths: 05-10, 20-25 and 50-55 cm. Samples were collected at each of the four corners of the plots and in their centres (see Kiely *et al.* 2009), giving a total of 15 samples for chemical analyses and another 15 samples for the determination of soil bulk density in each of the plots. To verify if there was any seasonal difference between winter and summer, we collected samples in January and July of 2016, with a total of 300 soil samples for each season and a total of 600 soil samples for the whole experiment. To reduce the effects of spatial variability within the plots, the data from each of the four corners and the centres of the plots were averaged (see Wellock *et al.* 2014). A hammer auger coupled with a metal ring of known volume (i.e., 88.62 cm<sup>3</sup>) was used to collect samples for soil bulk density, whilst a Dutch auger was used to collect samples for the chemical analyses. Soil bulk density

was determined by dividing the final weight by the initial volume after samples were dried until constant weight in a force draught oven at 70 °C.

### **Soil Chemical Analyses – Carbon and Nitrogen**

Soil carbon and nitrogen determinations were made on sieved soil samples (2 mm aperture), to remove stones and plant material, and then air dried before grinding them into a fine powder using a soil mill. Soil C and N were determined using a CHN elemental analyser (LECO Corporation ©, TruSpec) to estimate Total Carbon (TC) and Total Nitrogen (TN). Fresh soil samples were also extracted using 0.5 M K<sub>2</sub>SO<sub>4</sub> for determinations of Dissolved Organic Carbon (DOC) and Dissolved Organic Nitrogen (DON), using a TIC-TOC analyser (TOC-VCPH/TOC-VCPN Total Organic Carbon Analyser, Shimadzu Corporation ®, Analytical & Measuring, Instruments Division, Kyoto, Japan). Data on inorganic carbon was excluded from the analyses as the values were not detectable and assumed to be zero. To obtain data on soil nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>), fresh soil samples were extracted using 2M KCl and analysed using a flow injection analysis system (QuikChem ® Method 12-107-04-1-B and QuikChem ® Method 12-107-06-2-A, nitrate and ammonium, respectively, LACHAT Instruments, HACH Company ®). The analyses of nitrate and ammonium were performed only for the topsoil layer (05-10 cm) in January, April, July, and October, corresponding to winter, spring, summer, and autumn, respectively. To estimate soil TC and TN stocks, we multiplied the values for soil bulk density with their respective percentage of carbon and nitrogen given by the CHN elemental analyser, and then expressed the results on an area basis (Mg ha<sup>-1</sup>).

### **Soil DNA Extraction and Microbiome Profiling**

Soil bacterial community analyses were carried out on 18 samples collected during the summer, due to the poor recovery of DNA of sufficient quality from winter samples. The extraction of DNA from the soil was carried out using the DNeasy PowerSoil Pro kit (Qiagen, UK) following the manufacturers' instructions. The DNA concentration (ng/μl) and absorbance ratios (260:280 nm and 260:230 nm) were quantified by spectrophotometry using a ND-1000 Spectrophotometer (ThermoFisher, USA). Microbiome profiling was carried out by Eurofins



Genomics (Konstanz, Germany). The V3-V4 region of the bacterial 16S rRNA gene was amplified using the primers 357-F (TACGGGAGGCAGCAG; Turner *et al.* 1999) and 800R (CCAGGGTATCTAATCC; Kisand *et al.* 2002) and resulting amplicons were sequenced using the Illumina MiSeq platform. Amplicon sequences were processed using mothur v. 1.45.3 (Schloss *et al.* 2009). Sequences smaller than 200 bp, with 1 or more ambiguous nucleotides and homopolymers longer than 8 bp were removed from the dataset. Remaining sequences were aligned against the Silva reference alignment (Quast *et al.* 2013) and those sequences classified as plastid, mitochondrial, archaeal, eukaryotic, or that were unknown at the kingdom level, were also removed from the dataset. Chimeric sequences were detected using the vsearch algorithm tool within mothur (Rognes *et al.* 2016) and were then also removed. The sequences were classified to genus level in mothur using the in-built Ribosomal Database Project (RDP) sequence aligner (Schloss 2009). Relative abundances were determined as the abundance of each genus to the total number of sequences in a sample.

### **Determination of the Age of the Invasion**

Dating of the age of the invasions was carried out following the methodology of Fennell *et al.* (2014) on four soil cores collected from two different invasive stands at Dooega Beach. This technique is based on the germination of seeds from different soil layers that have been dated using  $^{137}\text{Cs}$ . Briefly, we collected soil cores, sliced them into 2 cm discs, put the discs into trays mixed with compost in a greenhouse and counted the numbers of seeds germinating. The last layer of the soil that had germinating seeds of *G. tinctoria* was correlated with the radiometric chronology to determine the age of the *G. tinctoria* invasion and its presence in the studied area. Full details and a critique of the approach are given in Fennell *et al.* (2014).

### **Statistical Analysis**

To compare seasonal differences (i.e., summer and winter) between soil bulk density, DOC, DON, soil TC and TN stocks amongst the two treatments, we used general linear mixed-model effects with restricted maximum likelihood, with uninvaded grasslands (GRASS) and areas invaded by *G. tinctoria* (GUN) as fixed factors and fields as random factors. We used the

same approach to check differences between the four seasons (winter, spring, summer, and autumn) regarding the concentrations of ammonium and nitrate for the topsoil layer (5-10 cm). To check for differences in isotopic compositions, Analysis of Variance (ANOVA) was applied. Bonferroni's *post-hoc* correction was applied after each test and visual analysis of the residuals was also carried out to ensure normality. These analyses were performed with a significance level of  $P = 0.05$ , using SPSS Statistics v. 24 (IBM 2016).

For assessments of changes the soil microbial community, multivariate statistical analysis was carried out in PRIMER v. 7.0.21 with PERMANOVA+ 1 add-on v. 1.0.1 (Quest Research, New Zealand). Potential differences in the microbial populations were also assessed using non-metric multidimensional scaling (NMDS), and permutational multivariate analysis of variance (PERMANOVA). Microbial diversity was checked using Shannon's H' Index. Datasets were square-root-transformed prior to their conversion into resemblance matrices based on the Bray-Curtis dissimilarity measure. The NMDS was performed on ranked dissimilarities using the Kruskal fit scheme 1. PERMANOVA tests were done by permutation of residuals under a reduced model using 9,999 permutations and ANOVA was carried out using SAS v. 9.4.

## Results and Discussion

### The Soil Food Web and *Gunnera tinctoria*

The leaves and roots of the GRASS plants had a similar isotopic composition, with mean ( $\pm$  SE)  $d^{13}C$  and  $d^{15}N$  values of  $-28.8\text{‰}$  (0.8) and  $3.21\text{‰}$  (1.1) in leaves and  $-29.0\text{‰}$  (0.7) and  $3.65\text{‰}$  (0.3) in roots (Fig. 1A). By contrast,  $d^{15}N$  was highly variable between *Gunnera* plant organs, spanning from  $3.2\text{‰}$  (0.6) in rhizomes to  $6.9\text{‰}$  (0.5) in leaves, with intermediate values ( $4.8\text{‰} \pm 0.8$ ) in the petiole. Contrary to expectations, there were no differences in  $d^{15}N$  between the grassland vegetation and the *G. tinctoria* samples, although the latter species can access atmospheric  $N_2$  through its symbiosis with *Nostoc*. No differences in soil  $d^{15}N$  between the two vegetation types were registered as well, possibly because of the presence of N-fixing legumes (e.g., *Trifolium* spp.) in the resident community (Mantoani *et al.* 2025). There was, however, a large spacing in the C isotope composition between the grass plants and any *G.*

*tinctoria* plant part ( $F_{1,13} = 60.69$ ;  $P > 0.001$ ), because *G. tinctoria* had much less negative  $\delta^{13}\text{C}$  values, ranging from to  $-25.6\text{‰}$  (0.2) in the rhizome to  $-24.5\text{‰}$  (0.5) in leaves (Fig. 1A).

Despite the long site colonisation history, the  $\delta^{13}\text{C}$  values of bulk soil organic matter had only shifted marginally from uninvaded grasslands ( $-28.0\text{‰} \pm 0.2$ ) towards less negative *G. tinctoria* values in invaded areas ( $-27.5\text{‰} \pm 0.2$ ), i.e., only a small amount of *Gunnera*-derived C was evident (Fig. 1A). This picture was in stark contrast to the isotopic composition of the detritivorous soil animals because the  $\delta^{13}\text{C}$  spacing in living plants was clearly reflected in all three taxa of worms ( $F_{2,27} = 15.31$ ;  $P = 0.00004$ ), irrespective of their feeding mode. Under *G. tinctoria*, the  $\delta^{13}\text{C}$  of the litter-feeding earthworm *L. rubellus* had shifted by  $1.9\text{‰}$  towards *Gunnera*, and in the soil-feeding *A. chlorotica*, it had shifted by  $1.8\text{‰}$ . In the tiny enchytraeid worms, the  $\delta^{13}\text{C}$  had even shifted by  $3.4\text{‰}$  (Fig. 1B).

The results of the isotopic analysis demonstrated that *G. tinctoria* is driving changes in the earthworm and enchytraeid community through trophic mechanisms, with an increased (i.e., 20-fold higher litter production) and higher quality (i.e., lower C:N ratio) food supply at its base, in comparison to uninvaded areas (Mantoani *et al.* 2022). There are many examples of invasive plant species that are shown to increase litter inputs (Ehrenfeld 2003; Wolkovich *et al.* 2010; Tamura & Tharayil 2014) and many have a higher biomass productivity than native species (Sakai *et al.* 2001; Bossdorf *et al.* 2005), with potentially similar consequences for the soil fauna. In the invaded areas we sampled there was a marked effect of *G. tinctoria* on earthworm populations, with more than a two-fold increase in biomass and an almost three-fold increase in earthworm abundance (Mantoani *et al.* 2022), reinforcing the idea that invasive plants can significantly modify the soil fauna (Reinhart & Callaway 2006; Zhang *et al.* 2019; Pearson *et al.* 2024). Whilst top invasive predators might disrupt trophic food webs (Wainright *et al.* 2021), plant invasions that produce large amounts of biomass and displace native plants, as *G. tinctoria* does (Gioria & Osborne 2013; Mantoani *et al.* 2020), appear to have the opposite effect, and favour some animal species (i.e., earthworms).

Measured above-ground litter accumulation at the invaded sites peaked at  $9.9 \text{ Mg DW}$  (i.e., dry weight)  $\text{ha}^{-1}$  (Mantoani *et al.* 2022). Using a range of reported feeding rates for litter-feeding earthworms (Curry & Schmidt 2007) of between 10 and 50 mg DW per g live earthworm biomass per day (and assuming 300 active days per year at the study site), we can estimate that the resident litter-feeding earthworm population (about  $60 \text{ g biomass m}^{-2}$ ) can consume between

1.8 and 9.0 Mg DW litter per year. These estimates suggest that the litter-feeding earthworm populations under *G. tinctoria* can consume a large proportion, if not most, of the litter produced in a year. Furthermore, the large spacing in  $\delta^{13}\text{C}$  observed in our study between *G. tinctoria* and grassland species of about 4‰ allowed us to infer the C sources of detritivorous worms in invaded areas. Under living *G. tinctoria*, the C isotopic composition of the two earthworm species and the much smaller enchytraeid worms had clearly shifted towards that of the invasive plant and away from that of the bulk soil organic matter. This suggests that *G. tinctoria* drives the soil food web by providing enhanced C inputs. Both litter and soil feeding worms species exhibited this shift, reflecting the fact that old, recalcitrant soil organic matter is of minor importance as a C source (Curry & Schmidt 2007). The isotope data nicely complement the earthworm population data from Mantoani *et al.* (2020) because they demonstrate a trophic link as a mechanism by which invasive plants can alter below-ground communities.

### **The Soil Bacterial Community and *Gunnera tinctoria* Invasions**

In total, 105 bacterial genera were detected in uninvaded plots and 121 were present in invaded areas. Alpha diversity was similar in both areas, with a Shannon H' Index of 2.55 in uninvaded areas and 2.27 in invaded areas. Based on the 20 most abundant genera in each area, the composition of the soil bacterial community under invaded stands was significantly different to that in uninvaded grasslands ( $P = 0.0001$ ) at genus level. Microbiome analysis revealed that, at genus level, the composition of the soil bacterial community in invaded areas was significantly different to that in uninvaded grasslands ( $P = 0.0001$ ) based on the most abundant genera. Soil depth also significantly affected bacterial community dynamics ( $P = 0.0032$ , Supplementary Table 1), with pair-wise analyses confirming that the community in invaded areas was different from that in the top soil layer of uninvaded areas (05-10 cm;  $t = 2.83$ ;  $P = 0.014$ ), for the middle layer (20-25 cm;  $t = 2.22$ ;  $P = 0.01$ ), and the deepest soil layer analysed (50-55 cm;  $t = 2.21$ ;  $P = 0.02$ ). The NMDS plot (Fig. 2) shows these differences and confirms that the soil bacterial community under invasive stands differed significantly from that found in the uninvaded plots, with the difference being maintained at each soil depth.

The dominant genus in both areas was *Bacillus*, accounting for approximately 22% of the genera detected (Table 1). Genera belonging to Acidobacteria groups 1, 2 and 3 were also

dominant in both areas, accounting for a combined *ca.* 35% of the genera detected in uninvaded areas and *ca.* 23% of the genera in invaded areas. The relative abundance of the genus *Nitrospira* was significantly higher under invasive stands than in uninvaded grasslands ( $P = 0.008$ ), accounting for  $7.32 \pm 1.38$  and  $2.94 \pm 0.46$  % of the sequences detected, respectively. Six bacterial genera (*Aciditerrimonas*, *Acidobacteria* Gp7, *Conexibacter*, *Defluviicoccus*, *Pseudonocardia*, and *Rhodomicrobium*) were among the 20 most abundant genera in uninvaded areas only. Conversely, a further six genera (*Acidobacteria* Gp5, *Acidobacteria* Gp13, *Hyphomicrobium*, *Nocardioides*, *Sporosarcina*, and genera belonging to candidate Phylum WS3) were among the most abundant genera in invaded plots only (Table 1). Changes in the abundance of methanotrophs were also found, with the *Methylocystaceae* family almost three-fold higher and *Methylobacter* four-fold lower in invaded areas in comparison to uninvaded grasslands. However, these changes were not significant due to the high variation between individual replicates.

Differences in soil bacterial communities under invasive stands have been reported previously (Reinhart & Callaway 2006; Gibbons *et al.* 2017; McTee *et al.* 2017) and our study reinforces the idea of invasive plants as drivers of soil bacterial communities. Although the majority of the 26 most abundant soil bacteria genera found in invaded areas were not significantly different from the uninvaded grasslands, there was a reduction in the abundance of six bacteria genera (*Aciditerrimonas*, *Acidobacteria* Gp7, *Conexibacter*, *Defluviicoccus*, *Pseudonocardia*, and *Rhodomicrobium*). In addition, the abundance of a further six soil bacteria genera (*Acidobacteria* Gp5, *Acidobacteria* Gp13, *Hyphomicrobium*, *Nocardioides*, *Sporosarcina*, and genera belonging to candidate Phylum WS3) increased in invaded areas (i.e., appeared in the top 20). Interestingly, the presence of this plant invader more than doubled the abundance of *Nitrospira*, which is usually involved in the oxidation of nitrite to nitrate (Daims & Wagner 2018).

Although the reason(s) for these differences are not known, both enchytraeids and endogeic earthworms obtain a significant proportion of their essential amino acids from soil bacteria (Larsen *et al.* 2016). Given the large increase in earthworms in invaded areas, particularly the endogeic forms (Mantoani *et al.* 2022), this could have resulted in a reduction in some bacterial genera. Many *Acidobacteria* (group 7) are thought to play a key role in the C, N and S biogeochemical cycles (Kalam *et al.* 2020) and this could reflect a reduced role in invaded

areas. Similarly, the generally significant role of the Actinomycetota in organic matter breakdown (Bao *et al.* 2021) may be associated with the increased involvement of earthworms in decomposition processes in invaded areas. The reduction in *Rhodomicrobium* in invaded areas could be because the abundance of this phototrophic bacterium is limited by the intense shade provided by the *G. tinctoria* canopy (Mantoani *et al.* 2020). The increases observed for the other bacteria genera, including *Nitrospira*, are more difficult to explain, but could be related to a general increase in easily metabolizable substrates provided by the decomposition of the large amounts of high-quality litter and/or are directly or indirectly associated with the more abundant earthworm populations.

A growing body of evidence suggests that *Nitrospira* is also involved in the COMAMMOX process, i.e., complete oxidation of ammonia to nitrate by a single organism (Daims *et al.* 2015; van Kessel *et al.* 2015). COMAMMOX *Nitrospira* are thought to be widely distributed (Shi *et al.* 2020), with some studies suggesting that COMAMMOX may be the dominant nitrification pathway in soils and other environments (Zhao *et al.* 2019; Hu *et al.* 2021). In the present study, Ammonia Oxidising Bacteria (AOB) and Nitrite Oxidising Bacteria (NOB) were poorly represented, with only 15 sequences related to *Nitrosomonas* (AOB) and 13 sequences of *Nitrobacter* (NOB) detected. No reads related to other AOB such as *Nitrosococcus*, *Nitrosospira* or *Nitrococcus* (AOB) were found. Approximately 13,500 sequences relating to *Nitrospira* were present, suggesting that complete ammonia oxidation may occur underneath invasive stands.

There is evidence that the soil bacterial genera that are promoted by *G. tinctoria* invasions, including *Nitrospira*, are linked to a higher N availability (Xu *et al.* 2020), and nitrate and DON accumulated in the topsoil layer in the current study. The factors underpinning these effects are often associated with higher quality litter inputs and the specific association between different invasive plants and their rhizosphere organisms, with potentially wide-ranging impacts for the whole ecosystem (Ehrenfeld 2003; Hawkes *et al.* 2005; Broadbent *et al.* 2017). The increase in soil pH during the summer under invasive stands (Mantoani & Osborne, 2022), can influence soil TC/TN stocks (Jiao *et al.* 2016) and nitrification/denitrification processes, leading to higher soil nitrate levels (Šimek & Cooper 2002), which was observed in the current study. All these effects can lead to modifications in the composition and activity of the soil bacterial community (Che *et al.* 2015), favouring genera/species that are linked to high N availability in

invaded areas. In addition to the observed changes in bacteria associated with soil N transformations we also found evidence for alterations in the abundance of methanotrophs, indicating that there may be wider impacts on the soil microbial community than those associated with N-related metabolic pathways.

### Soil Physio-chemical Properties and *G. tinctoria* Invasion

Soil bulk density increased with depth ( $F_{(2,48)} = 10.15$ ,  $P < 0.001$ ), but was not significantly different between invaded and uninvaded areas ( $F_{(2,48)} = 0.003$ ,  $P = 0.997$ ) for any season studied (Supplementary Table 2). No differences in DOC were found between the two treatments ( $F_{(2,48)} = 0.067$ ,  $P = 0.935$ ; Fig. 3A). The levels of DON, however, decreased with soil depth in both treatments ( $F_{(2,48)} = 80.38$ ,  $P < 0.001$ ) and was higher in the *G. tinctoria* plots in the near-surface layer (05-10 cm;  $F_{(1,48)} = 8.99$ ,  $P = 0.004$ ; Fig. 3B). Differences in DON were up to 50% higher (38.06 mg N kg soil<sup>-1</sup>) in invaded areas (CI = 58.88, 93.94 and 96.94, 131.99, uninvaded grasslands and invaded areas, respectively).

Together with an increase in DON in the near-surface layers under invasive stands, we also found an increase in nitrate ( $F_{(3,24)} = 6.65$ ;  $P = 0.002$ ) throughout the year, which elevated the soil concentrations by 6.40 mg N kg soil<sup>-1</sup> (95% CI = 0.25, 12.56) in summer and by 8.73 mg N kg soil<sup>-1</sup> (95% CI = 2.58, 14.89) in autumn, in comparison to uninvaded plots (Fig. 4B). Concomitantly, there was a reduction in ammonium levels ( $F_{(3,24)} = 12.63$ ;  $P < 0.001$ ) throughout the year (Fig. 4A), with uninvaded grasslands showing a decrease of 11.42 mg N kg soil<sup>-1</sup> (95% CI = 4.40, 18.45) and invaded plots a decrease of 9.19 mg N kg soil<sup>-1</sup> (95% CI = 2.17, 16.22). There was an increase in soil TC stocks with soil depth ( $F_{(2,48)} = 3.51$ ,  $P = 0.038$ ), but no significant differences were found between treatments ( $F_{(1,48)} = 0.293$ ,  $P = 0.591$ ) or seasons ( $F_{(1,48)} = 0.0004$ ,  $P = 0.984$ ; Supplementary Fig. 1A). No differences in soil TN stocks were found amongst treatments ( $F_{(1,48)} = 0.222$ ;  $P = 0.640$ ), seasons ( $F_{(1,48)} = 1.42$ ;  $P = 0.239$ ) or soil layers ( $F_{(2,48)} = 0.205$ ;  $P = 0.816$ ; Supplementary Fig. 1B).

It is important to note that the increased numbers of earthworms underneath the canopy of *G. tinctoria* could have an impact in altering soil C and N as described before (Bohlen *et al.* 2004) and could also contribute to shaping the soil bacterial community, which, in turn, could alter the soil nutrient balance. As illustrated previously (de Graaff *et al.* 2015), interactions

between earthworms and soil bacteria contribute to soil TC and TN stocks. These could augment the loss of nutrients under invasive stands, reducing any potential differences in soil physiochemistry attributes between uninvaded and invaded sites, and indicating that higher plant productivity does not necessarily translate into higher soil TC and TN stocks. Consequently, invasive alien plants that produce a higher quality (low C:N ratio) litter with faster decomposition rates may not necessarily increase carbon stocks, due to a loss of soil C possibly through microbial priming effects (Tamura & Tharayil 2014). This could reflect the situation in invaded areas where the previous recalcitrant grassland litter inputs have been replaced by more accessible and faster decomposing material supplied by *G. tinctoria* (Mantoani *et al.* 2022). It further suggests that invasive plants may not always induce any major changes in soil properties (Stefanowicz *et al.* 2017), if at the same time they modify the soil biota (Stefanowicz *et al.* 2016; Pearson *et al.* 2024).

### **Significance of the Age of the Invasion and the Generality of the Impacts**

Based on the seed germination results from different soil layers, dated using  $^{137}\text{Cs}$ , the age of the invasion was estimated at 107 years and 49 years for the first and the second soil cores, respectively. The deepest soil layers at which seed germination occurred were 25 and 27 cm for the first and the second core, respectively. The chronologies were also consistent with the  $^{137}\text{Cs}$  profiles for both soil cores. Thus, the date for the first appearance of significant *G. tinctoria* populations that were examined in the current study was approximately 110 years and 50 years, for the first and second soil core, respectively. One of the problems associated with many assessments of the ecosystem impact of introduced plants is the uncertainty about the duration of the invasion, which could have a major impact on the results obtained (Flory *et al.* 2017).

Based on the estimates of the age of the different populations in this (49-107 years) and a previous study (70-100 years; Fennell *et al.* 2014), the populations we studied had an average age of > 80 years. The variability in the estimates could be due to variations in colonisation, growth, and development, which could vary with site and environmental conditions. Whilst these results indicate that long lived (> 49 years) *G. tinctoria* populations have an altered detrital food chain, it is unclear how long it takes, from the initial establishment of introduced populations, to result in these modifications and how reversible they might be (i.e., presence of legacy effects).



Previous results involving the removal or herbicide treatment of mature *G. tinctoria* populations have shown that earthworm numbers can return to within 30% of those in uninvaded areas within one year (Mantoani *et al.* 2022), confirming that the increase in earthworm populations is largely dependent on the current year's litter production.

These results show that once a significant pool of litter is produced, continuous annual inputs are required to maintain the worm populations and associated alterations in the detrital food chain. Of particular significance is the development of a large rhizome as once this is formed annual above-ground productivity is high (Mantoani *et al.* 2020). A conservative estimate of how long this takes, based on personal observations, could be of the order of 10-20 years, an age that is significantly less than the populations examined in the current study, when sufficient litter may be present to result in a significant change in the worm population, but further work would be required to confirm this. Moreover, the generality of these effects is also unclear. In New Zealand, invasive broom (a leguminous N-fixing shrub) was associated with larger populations of (undetermined) Oligochaeta (Pearson *et al.* 2024). On the other hand, *Rhododendron ponticum* invasions led to the disappearance of most of the earthworm populations in two independent studies in the UK and Ireland (Clare 2016; Melody & Schmidt 2021). Whilst the reason(s) for these differences are not known, the lower quality litter produced by *R. ponticum* (C:N = 45:1) compared to *G. tinctoria* (C:N = 17:1) may be a factor (Mantoani *et al.* 2022). Similarly, the lower C:N ratio in the uninvaded grassland areas used in the current study (C:N = 29:1) may also contribute to the lower earthworm populations independently of differences in litter production.

The evidence presented in this work expands on previous studies by identifying trophic mechanisms that explain why *G. tinctoria* invasions can have wide-ranging and contrasting impacts on ecosystems, including detrimental, or neutral effects (Mantoani *et al.* 2020; Mantoani & Osborne 2022) as well as beneficial or positive effects (Mantoani *et al.* 2022; Mantoani & Osborne 2021). The current study shows that *G. tinctoria* invasions can result in a shift in the soil biota and modifications in the detrital food chain, with an increased role for earthworms in decomposition processes. Overall, these results indicate the complexity of impacts that an invasive species may have on ecosystems and ecosystem processes, highlighting the need for more work that encompasses a range of invasive species to assess the generality of these results.

**Acknowledgements:** The authors thank the support received from local landowners and Mayo County Council. We also thank Mr. Eugene Sherry and Mrs. Cristina Motta Pechin who gave exceptional help in the field, Mr. Darragh Furey for technical assistance and sample preparation, and Marie and Martin McGreal for their hospitality and lovely accommodation.

**Funding:** MCM was supported by the Brazilian National Council for Scientific and Technological Development (CNPq; grant number 205031/2014-5).

**Competing Interests:** The author(s) declare none.

**Data Availability:** Data will be made available upon request.

## References

Bao Y, Dolfing J, Guo Z, Chen R, Meng W, Li Z, Lin X, Feng Y (2021) Importance ecophysiological roles of non-dominant Actinobacteria in plant residue decomposition, especially in less fertile soils. *Microbiome*, 9: 84

Bohlen PJ, Scheu S, Hale CM, McLean MA, Migge S, Groffman PM, Parkins D (2004) Non-native invasive earthworms as agents of change in northern temperate forests. *Frontiers in Ecology and the Environment* 2: 427-435

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

Broadbent AAD, Orwin KH, Peltzer DA, Dickie IA, Mason NWH, Ostle NJ, Stevens CJ (2017) Invasive N-fixer impacts on litter decomposition driven by changes to soil properties not litter quality. *Ecosystems* 20: 1151-1163

Che J, Zhao XQ, Zhou X, Jia ZJ, Shen RF (2015) High pH-enhanced soil nitrification was associated with ammonia-oxidizing bacteria rather than archaea in acidic soils. *Applied Soil Ecology* 85: 21-29

Clare M (2016) The Impact of *Rhododendron ponticum* as an Invasive Species on Ecosystem Functions of Lancaster University Campus. *Weberbauerella* 1: 1-11

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

Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH (2015) Complete nitrification by *Nitrospira* bacteria. *Nature* 528: 504-509

Daims H, Wagner M (2018) *Nitrospira*. *Trends in Microbiology* 26: 462-463

Diden WAM (1993) Ecology of terrestrial Enchytraeidae. *Pedobiologia* 37: 2-29

de Graaff M-A, Adkins J, Kardol P, Throop HL (2015) A meta-analysis of soil biodiversity impacts on the carbon cycle. *SOIL* 1: 257-271

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. *Annual Review of Ecology Evolution and Systematics* 41: 59-80

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

Flory SL, Bauer J, Phillips RP, Clay K (2017) Effects of a non-native grass invasion decline over time. *Journal of Ecology* 105: 1475-1484

Gibbons SM, Lekberg Y, Mummey DL, Sangwan N, Ramsey PW, Gilbert JA (2017) Invasive plants rapidly reshape soil properties in a grassland ecosystem. *mSystems* 2: e00178-16

Gioria M, Osborne B (2013) Biological flora of the British Isles: *Gunnera tinctoria*. *Journal of Ecology* 101: 243-264

Gioria M, Carta A, Balogianni V, Fornara D, Pyšek P, Osborne BA (2023) Changes in the functional and phylogenetic diversity of above- and below-ground plant communities invaded by two alien herbs. *Neobiota* 88: 75-101

Griffiths HM, Ashton LA, Parr CL, Eggleton P (2021) The impact of invertebrate decomposers on plants and soil. *New Phytologist* 231: 2142-2149

Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters* 8: 976-985

Hu J, Yuxiang Z, Yao X, Wang J, Zheng P, Xi C, Hu B. (2021) Dominance of COMAMMOX *Nitrospira* in soil nitrification. *Science of The Total Environment* 780: 146558

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

Jang J, Xiong X, Liu C, Yoo K, Ishii S (2022) Invasive earthworms alter forest soil microbiomes and nitrogen cycling. *Soil Biology and Biochemistry* 171: 108724

Jiao F, Shi X, Han, F, Yuan Z (2016) Increasing aridity, temperature and soil pH induce soil C-N-P imbalance in grasslands. *Scientific Reports* 6: 19601

Kalam S, Basu A, Ahjmad I, Sayyed RZ, El-Enshasy HA, Dailin DJ, Surian NL (2020) Recent understanding of soil acidobacteria: a critical review. *Frontiers in Microbiology* 11: 580024

Kisand V, Cuadros R, Wikner J (2002) Phylogeny of Culturable Estuarine Bacteria Catabolizing Riverine Organic Matter in the Northern Baltic Sea. *Applied and Environmental Microbiology* 68: 379-388

Kiely G, McGoff NM, Eaton JM, Xu X, Leahy P, Carton O (2009) SoilC - Measurement and modelling of soil carbon stocks and stock changes in Irish soils. STRIVE Report Series No. 35. Prepared for the Environmental Protection Agency (EPA) STRIVE Programme 2007-2013

Larsen, T, Pollierer, MM, Holmstrup, M, D'Annibale, A, Maraldo, K, Andersen, Eriksen, J (2016) Substantial nutritional contribution of bacterial amino acids to earthworms and enchytraeids: a case study from organic grasslands. *Soil Biology and Biochemistry* 99: 21-27

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

Mantoani MC, Osborne BA (2021a) Alien plant introductions and greenhouse gas emissions: insights from *Gunnera tinctoria* invasions. *Science of the Total Environment* 775: 145861

Mantoani MC., Osborne BA (2021b) The effect of cutting and waterlogging on plant-related CO<sub>2</sub> and N<sub>2</sub>O fluxes associated with the invasive N-fixing species *Gunnera tinctoria*. *Diversity* 13: 427

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, Alhakami FT, Fearon H, Gioria M, Schmidt O, Osborne B (2022) *Gunnera tinctoria* invasions increase, not decrease, earthworm abundance and diversity. *Biological Invasions* 24: 3721-3734

Mantoani MC, Sweeney C, Osborne BA (2025) Complex and dynamic effects of an extreme low temperature weather event on invasive plant populations and resident communities. *Global Change Biology*: in press.

Mary B, Recous S, Darwis D, Robin D (1996) Interactions between decomposition of plant residues and nitrogen cycling in soil. *Plant and Soil* 181, 71-82

McTee MR, Lekberg Y, Mummey D, Rummel A, Ramsey PW (2017) Do invasive plants structure microbial communities to accelerate decomposition in intermountain grasslands? *Ecology and Evolution* 7: 11227-11235

Medina-Sauza RM, Álvarez-Jiménez M, Delhal A, Reverchon F, Blouin M, Guerrero-Analco JA, Cerdán CR, Guevara R, Villain L, Barois I (2019) Earthworms building up soil microbiota, a review. *Frontiers in Environmental Science* 7: 81

Melody C, Schmidt O (2021) A terrestrial annelid (earthworms) survey in woodland, grassland and garden habitats of Killarney National Park, Co. Kerry. Unpublished report for the National Parks and Wildlife Service (NPWS), Dublin, Ireland

Osborne B, Bergman B (2009) Why does *Gunnera* do it and other Angiosperms don't? An evolutionary perspective on the *Gunnera-Nostoc* symbiosis. In Pawlowski K (ed). *Prokaryotic Symbionts in Plants (Microbiology Monographs)*, Vol. 8. Heidelberg, Germany: Springer, 207-224

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

Osborne BA, Sprent JI (2002) Ecology of the *Nostoc-Gunnera* symbiosis. In Rai AN, Bergman B, Rasmussen U (eds). *Cyanobacteria in Symbiosis*. Dordrecht, The Netherlands: Springer, 233-251

Pearson BM, Minor MA, Robertson AW, Clavijo-McCormick AL (2024) Plant invasion down under: exploring the below-ground impact of invasive plant species on soil properties and invertebrate communities in the Central Plateau of New Zealand. *Biological Invasions* 26: 4215-4228

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41(D1): D590-D596

Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. *New Phytologist* 170: 445-457

Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4: e2584

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. *Annual Review of Ecology and Systematics* 32: 305-332

Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. *Annals of Botany* 111: 743-767

Schloss PD (2009) A High-Throughput DNA Sequence Aligner for Microbial Ecology Studies. *PLOS ONE* 4: e8230

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75: 7537-41

van Kessel M, Speth D, Albertsen M, Nielsen PH, op den Camp HJM, Kartal B, Jetten MSM, Lückner S (2015) Complete nitrification by a single microorganism. *Nature* 528: 555-559

Shi Y, Jiang Y, Wang S, Wang X, Zhu G (2020) Biogeographic distribution of COMAMMOX bacteria in diverse terrestrial habitats. *Science of The Total Environment* 717: 137257

Shutenko GS, Andriuzzi WS, Dyckmans J, Luo Y, Wilkinson TL, Schmidt O (2022) Rapid transfer of C and N excreted by decomposer soil animals to plants and above-ground herbivores. *Soil Biology and Biochemistry* 166: 108582

Šimek M, Cooper JE (2002) The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science* 53: 345-354

Stefanowicz AM, Stanek M, Nobis M, Zubek S (2016) Species-specific effects of plant invasions on activity, biomass, and composition of soil microbial communities. *Biology and Fertility of Soils* 52: 841-852

Stefanowicz AM, Stanek M, Nobis M, Zubek S (2017) Few effects of invasive plants *Reynoutria japonica*, *Rudbeckia laciniata* and *Solidago gigantea* on soil physical and chemical properties. *Science of the Total Environment* 574: 938-946

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

Turner S, Pryer KM, Miao VPW, Palmer JD (1999) Investigating Deep Phylogenetic Relationships Among Cyanobacteria and Plastids by Small Subunit rRNA Sequence Analysis. *Journal of Eukaryotic Microbiology* 46: 327-338

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

Wainright CA, Muhlfeld CC, Elser JJ, Bourret SL, Devlin SP (2021) Species invasion progressively disrupts the trophic structure of native food webs. *PNAS* 118: e2102179118



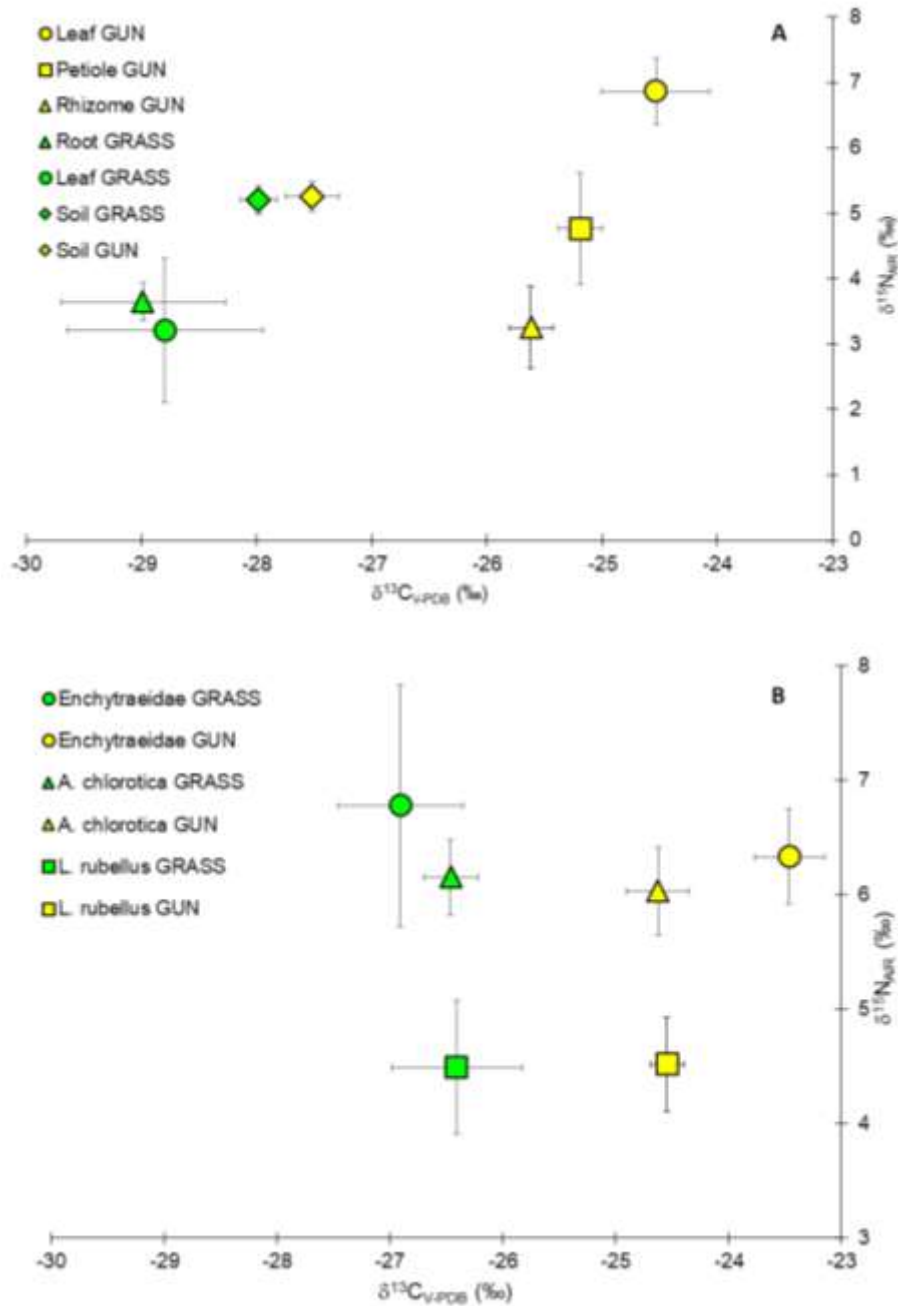
Wellock ML, Rafique R, LaPerle1 CM, Peichl M, Kiely G (2014) Changes in ecosystem carbon stocks in a grassland ash (*Fraxinus excelsior* L.) afforestation chronosequence in Ireland. *Journal of Plant Ecology* 7: 429-438

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. *Global Change Biology* 16: 1351-1365

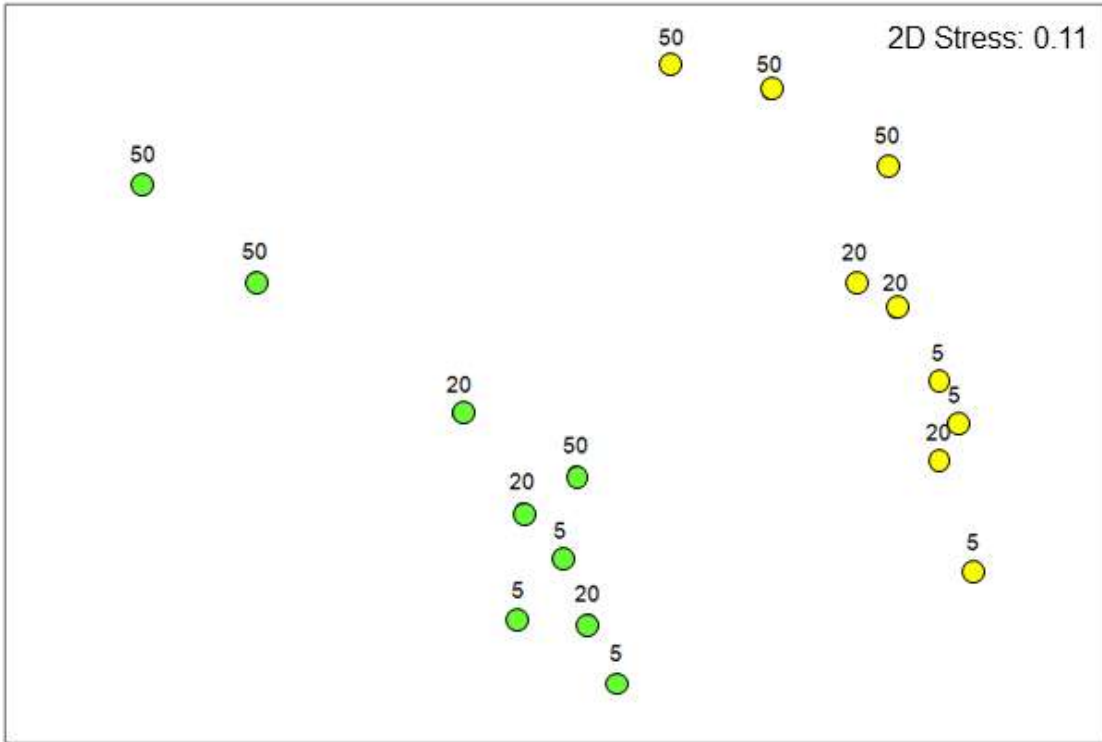
Xu S, Wang B, Li Y, Jiang D, Zhou Y, Ding A, Zong Y, Ling X, Zhang S, Lu H (2020) Ubiquity, diversity, and activity of COMAMMOX *Nitrospira* in agricultural soils. *Science of the Total Environment* 706: 135684

Zhang P, Li B, Wu J, Hu S (2019) Invasive plants differentially affect soil biota through litter and rhizosphere pathways: a meta-analysis. *Ecology Letters* 22: 200-210

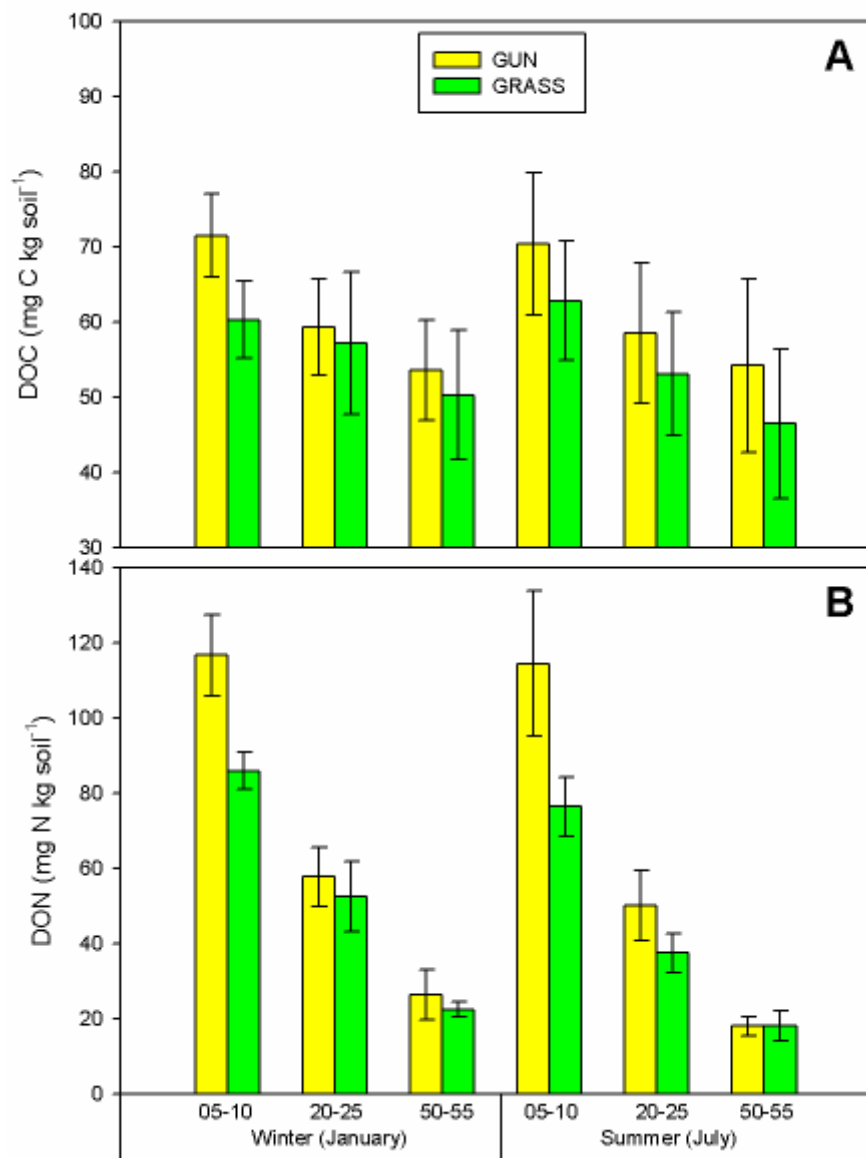
Zhao Z, Huang G, He S, Zhou N, Wang M, Dang C, Wang J, Zheng M (2019) Abundance and community composition of COMAMMOX bacteria in different ecosystems by a universal primer set. *Science of The Total Environment* 691: 146-155



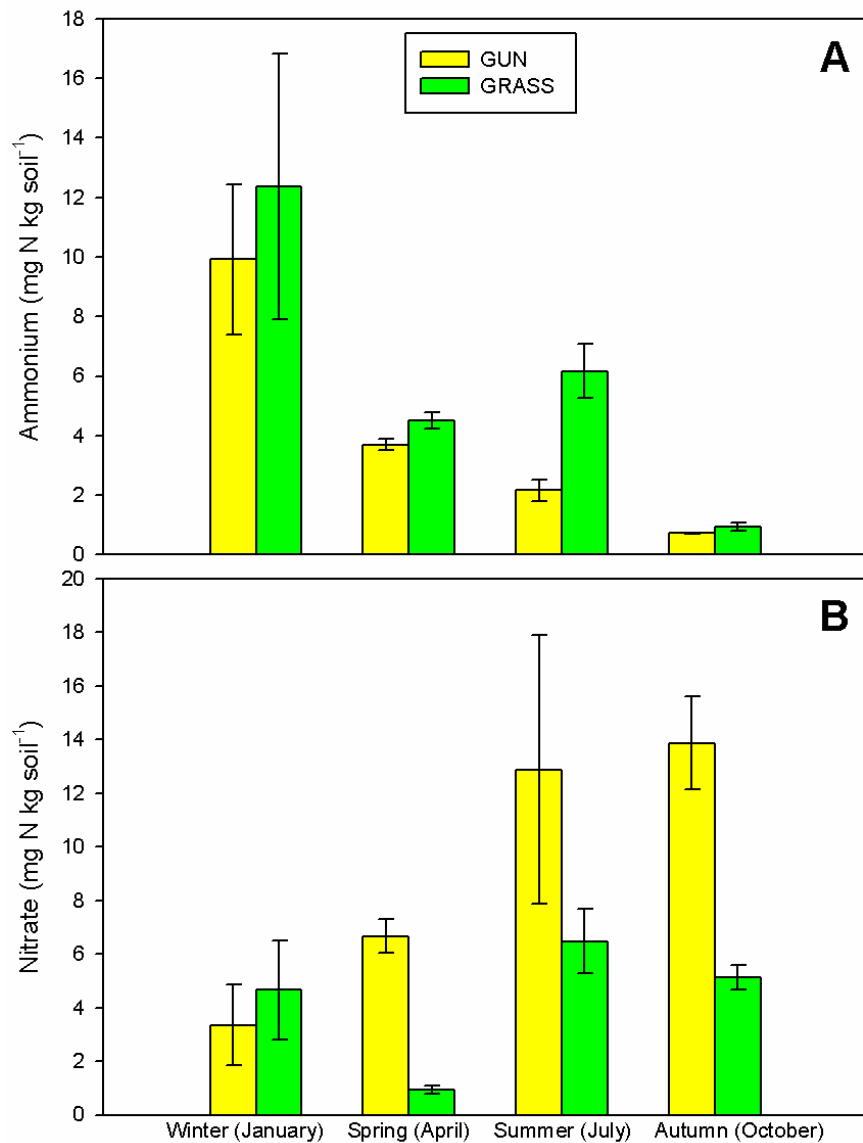
**Figure 1** – Biplots of the carbon and nitrogen isotope composition of (A) plant parts and soil, and (B) enchytraeid worms and two earthworm species collected on Achill Island, Co. Mayo, Ireland. Legend: GRASS = uninvasion semi-natural grasslands (n = 9, 5 and 6, earthworms, soil, and plant parts, respectively; mean  $\pm$  1SE); GUN = areas invaded by *Gunnera tinctoria* (n = 11, 5 and 9, earthworms, soil, and plant parts, respectively; mean  $\pm$  1SE).



**Figure 2** – Non-metric multidimensional scaling (NMDS) plot for the soil bacterial community present in uninvaded grasslands (GRASS = green dots) and areas invaded by *Gunnera tinctoria* (GUN = yellow dots) (n = 9), on Achill Island, Co. Mayo, Ireland. Soil depth is indicated by numbers above the symbols (5 = 05-10 cm; 20 = 20-25 cm; 50 = 50-55cm). Note: this analysis considers only soil samples collected during the summer due to the low retrieval of DNA in the winter.



**Figure 3** – Seasonal variation in (A) dissolved organic carbon (DOC, mg C kg soil<sup>-1</sup>) and in (B) dissolved organic nitrogen (DON, mg N kg soil<sup>-1</sup>) during 2016, according to the three soil depths analysed (05-10, 20-25, and 50-55 cm), on Achill Island, Co. Mayo, Ireland (n = 5; mean ± 1SE). Legend: GRASS = uninvasion semi-natural grasslands; GUN = areas invaded by *Gunnera tinctoria*.



**Figure 4** – Seasonal variation in (A) ammonium (mg N kg soil<sup>-1</sup>) and in (B) nitrate (mg N kg soil<sup>-1</sup>) in the topsoil layer (05-10 cm) throughout 2016, on Achill Island, Co. Mayo, Ireland (n = 5; mean ± 1SE). Legend: GRASS = uninvaded semi-natural grasslands; GUN = areas invaded by *Gunnera tinctoria*.

**Table 1** – Relative abundance (%) of the 20 most abundant bacterial genera in uninvaded semi-natural grasslands (GRASS) and areas invaded by *Gunnera tinctoria* (GUN)  $\pm$  1SE. Legend: \* indicates that a genus was not among the top 20 most abundant bacterial genera in a particular treatment.

<b>Bacterial Genus</b>	<b>GRASS</b>	<b>GUN</b>
<i>Bacillus</i>	22.64 $\pm$ 5.77	23.64 $\pm$ 4.93
<i>Acidobacteria Gp3</i>	14.71 $\pm$ 2.24	9.26 $\pm$ 0.97
<i>Acidobacteria Gp1</i>	11.71 $\pm$ 3.27	7.15 $\pm$ 2.79
<i>Acidobacteria Gp2</i>	8.34 $\pm$ 2.77	6.94 $\pm$ 2.17
<i>Clostridium_sensu_stricto</i>	5.08 $\pm$ 0.73	5.98 $\pm$ 0.74
<i>Mycobacterium</i>	4.35 $\pm$ 1.39	2.29 $\pm$ 0.51
<i>Ilumatobacter</i>	3.28 $\pm$ 1.16	3.86 $\pm$ 1.3
<i>Nitrospira</i>	2.94 $\pm$ 0.46	7.32 $\pm$ 1.38
<i>Acidobacteria Gp13</i>	*	5.48 $\pm$ 3.81
<i>Geminicoccus</i>	2.76 $\pm$ 0.73	2.71 $\pm$ 0.49
<i>Acidobacteria Gp7</i>	3.15 $\pm$ 0.65	*
<i>Rhodomicrobium</i>	2.17 $\pm$ 0.44	*
<i>Defluviicoccus</i>	2.13 $\pm$ 0.75	*
<i>Gemmatimonas</i>	2.60 $\pm$ 0.48	1.94 $\pm$ 0.22
<i>Paenibacillus</i>	2.15 $\pm$ 0.35	3.35 $\pm$ 0.58
<i>Pedomicrobium</i>	3.19 $\pm$ 0.95	4.03 $\pm$ 1.3
<i>Bradyrhizobium</i>	2.09 $\pm$ 0.69	2.42 $\pm$ 0.79
<i>Pseudonocardia</i>	1.87 $\pm$ 0.44	*
<i>Conexibacter</i>	1.56 $\pm$ 0.46	*
<i>Hyphomicrobium</i>	*	2.87 $\pm$ 0.85
<i>Aciditerrimonas</i>	1.75 $\pm$ 0.39	*
<i>Acidobacteria Gp5</i>	*	2.30 $\pm$ 0.41
<i>WS3_genus_incertae_sedis</i>	*	2.46 $\pm$ 0.61
<i>Clostridium_XI</i>	1.52 $\pm$ 0.68	3.05 $\pm$ 0.77
<i>Nocardioides</i>	*	1.54 $\pm$ 0.46
<i>Sporosarcina</i>	*	1.37 $\pm$ 0.5