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Invasive earthworms modulate native plant trait expression and competition

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Biological invasions have major impacts on a variety of ecosystems and threaten native biodiversity. Earthworms have been absent from northern parts of North America since the last ice age, but non-native earthworms were recently introduced there and are now being spread by human activities. While past work has shown that plant communities in earthworm-invaded areas change towards a lower diversity mainly dominated by grasses, the underlying mechanisms related to changes in the biotic interactions of the plants are not well understood. Here, we used a trait-based approach to study the effect of earthworms on interspecific plant competition and aboveground herbivory. We conducted a microcosm experiment in a growth chamber with a full-factorial design using three plant species native to northern North American deciduous forests, *Poa palustris* (grass), *Symphyotrichum laeve* (herb) and *Vicia americana* (legume), either growing in monoculture or in a mixture of three. These plant community treatments were crossed with earthworm (presence or absence) and herbivore (presence or absence) treatments. Eight out of the fourteen above- and belowground plant functional traits studied were significantly affected by earthworms, either by a general effect or in interaction with plant species identity, plant diversity level and/or herbivore presence. Earthworms increased the aboveground productivity and the number of inflorescences of the grass *P. palustris*. Further, earthworms and herbivores together affected root tissue density of *P. palustris* and the specific leaf area of *V. americana*. In this study, earthworm presence gave a competitive advantage to the grass species *P. palustris* by inducing changes in plant functional traits. Our results suggest that invasive earthworms can alter competitive and multitrophic interactions of plants, shedding light on some of the mechanisms behind invasive earthworm-induced plant community changes in northern North America forests.

Keywords: biological invasion, competition, detritivore, herbivory, plant functional traits

Introduction

Biological invasions occur globally and are among the most significant direct causes of native biodiversity loss worldwide (Ehrenfeld 2010, Murphy and Romanuk 2014, Díaz et al. 2019). Dramatic changes in biodiversity can be due to invasive species acting as ecosystem engineers, such as European earthworms in northern North America (Bohlen et al. 2004a, Ehrenfeld 2010, Blouin et al. 2013, Ferlian et al. 2018). Much of the native flora and fauna was eradicated in this region during the last ice age, including earthworms (Gates 1982), which have been reintroduced by European settlers within the last centuries (Hendrix and Bohlen 2002).

In areas where invasive earthworms are abundant, they alter the physico-chemical properties of the soil (Bohlen et al. 2004a, Hale et al. 2005, Ferlian et al. 2020). The litter coverage is reduced, soil horizons are partly homogenized, nutrients redistributed and soil pH is increased while soil water content is decreased (Hale et al. 2005, Eisenhauer et al. 2007, Blouin et al. 2013, Ferlian et al. 2020). These modifications of abiotic conditions alter the habitat for soil-living organisms (Mclean and Parkinson 2000, Eisenhauer et al. 2007): soil community composition was shown to be significantly altered by invasive earthworms, with earthworm invasion decreasing the diversity and density of soil microbes and soil invertebrates (Eisenhauer et al. 2007, Ferlian et al. 2018).

Further, earthworms can act as an ecological filter on seeds, seedlings and plants (Eisenhauer and Scheu 2008a, Forey et al. 2011, Eisenhauer et al. 2012), impacting plant species establishment. The diversity of understory plants was reported to decrease, and the species and functional community composition changed significantly in response to the invasion of earthworms (Bohlen et al. 2004b, Hale et al. 2006, Craven et al. 2017). However, it was also shown that there are winners and losers: while some grass and non-native plant species benefit from earthworm invasion (Craven et al. 2017), the richness and abundance of herbs (Hale et al. 2006) and legumes (Eisenhauer et al. 2007) decrease in invaded forests, leading in some cases to sparse vegetation with only few herbaceous plant species left (Bohlen et al. 2004b). Nevertheless, the total plant cover was overall shown to increase and be dominated by graminoids (Craven et al. 2017), indicating that earthworms may change competitive interactions by benefitting certain plant species (Wurst et al. 2005, Eisenhauer and Scheu 2008b, Nuzzo et al. 2009). However, the mechanisms behind these changes and grass species dominance are understudied. In this context, our study aims to disentangle the mechanisms underlying plant community changes due to earthworm invasion, by focusing on biotic interactions and changes in plant functional traits sensu Violle et al. (2007).

The provision of available nitrogen is considered as the main pathway of earthworms to affect plants (Van Groenigen et al. 2014). Earthworms enhance the nitrogen uptake of grasses, herbs and legumes from organic litter and soil (Wurst et al. 2003, Eisenhauer and Scheu 2008b). Furthermore, their effects on soil structure, microbiota and water availability can also influence plant development (Scheu 2003, Eisenhauer et al. 2012, Blouin et al. 2013, Cameron et al. 2014). Overall, grass species would be favored under earthworm invasion due to their high tolerance to drought and ability to germinate on bare soil (Eisenhauer et al. 2012), but also because they are considered as fast-growing plants (Reich 2014) that efficiently exploit nutrient patches by building more root biomass and taking up more nitrogen when earthworms are present (Wurst et al. 2005, Eisenhauer and Scheu 2008b), in contrast to slow-growing herbs (Lavorel et al. 2007, Linder et al. 2018). Despite variations across plant species and functional groups, earthworms were shown to affect various plant functional traits related to the development and resource uptake (Scheu 2003, Van Groenigen et al. 2014, Blume-Werry et al. 2020, Thouvenot et al. 2021), but also to plant reproduction and defenses (Poveda et al. 2005, Thakur et al. 2021, Thouvenot et al. 2021). For example, some species with a more flexible rooting strategy were shown to be able to better adapt to the presence of invasive earthworms and forage in their nutrient-rich burrows (Cameron et al. 2014), which can give them a competitive advantage over their neighbor. Moreover, the leaf nitrogen content, which is considered as one of the most important plant traits to explain herbivory increase in grassland (Loranger et al. 2012), was shown to be enhanced by earthworm activity (Newington et al. 2004, Wurst et al. 2004, Xiao et al. 2018, Blume-Werry et al. 2020). Conversely, leaf dry matter content (LDMC), a trait that could be used as a proxy for leaf toughness and herbivore resistance (Elger and Willby 2003), showed inconsistent responses to the presence of earthworms (Thakur et al. 2021, Thouvenot et al. 2021).

By having dissimilar effects on plant functional traits of different species, invasive earthworms can affect the outcomes of biotic interactions of plants, e.g. by changing competition and facilitative interactions, and/or multitrophic interactions (Eisenhauer et al. 2009a, Wurst 2010, Blouin et al. 2013, Xiao et al. 2018). For example, it was shown that legumes produced less biomass when growing with grasses in the presence of earthworms (Eisenhauer and Scheu 2008b), which indicates a strong competitive disadvantage that might explain the lower legume cover and biomass observed in earthworm-invaded aspen forests (Eisenhauer et al. 2007). In addition, earthworms can also affect aboveground herbivory through plant-mediated effects positively (Newington et al. 2004, Thakur et al. 2021), or negatively (Wurst et al. 2003, Zaller et al. 2013), with the outcomes of this interaction probably depending on the plant and herbivore species. Leaf-chewing insects (Thakur et al. 2021) and deer (Dobson and Blossey 2015) that native understory plants face in previously earthworm-free regions will probably not consume plant species with similar traits, albeit they both are expected to increase their herbivory pressure on the most palatable species (i.e. plant with a high nitrogen content) in the presence of earthworms (Frelich et al. 2006, Dobson and Blossey 2015, Thakur et al. 2021).

To improve the mechanistic understanding of the effects of invasive earthworms on native understory plant communities in northern North American forests (Craven et al. 2017), we set up a microcosm experiment in a growth chamber with plants belonging to three functional groups (grass, herb and legume), either in monoculture or a mixture. Each plant community was subjected to the presence and absence of earthworms for ~ 12 weeks and aboveground herbivores for five days, respectively, resulting in a full-factorial design. To shed light on potential treatment effects, we assessed a set of plant functional traits that are likely to play critical roles in plant resource use, competition and responses to herbivores. As earthworms were expected to increase nutrient availability for plants, we hypothesized that 1) invasive earthworms increase overall productivity of plant species. Given that grasses can exploit soil nutrients rapidly (Linder et al. 2018) and their response to invasive earthworms is more pronounced than that of herbs (Thouvenot et al. 2021), we hypothesized that 2) the grass species becomes a stronger competitor and shows increased growth, while the herb and legume show decreased growth due to high competition with the grass species in the mixture and in the presence of earthworms. Moreover, as herbivory can affect plant traits (Heinze 2020), we expected 3) potential short-term effects of aboveground herbivory (five days) on plant traits (Poveda et al. 2003), while 4) invasive earthworms may modulate herbivory effects due to changes in soil nutrient availability (Eisenhauer and Scheu 2008b). Further, we expected that 5) the effects of invasive earthworms and aboveground herbivory depend on plant community composition, e.g. with the grass benefitting most from the presence of earthworms and herbivores in plant mixtures (Eisenhauer and Scheu 2008b).

Material and methods

Experimental set-up

Earthworm effects on biotic interactions (e.g. competition and herbivory) were studied in a microcosm experiment under controlled conditions using a full-factorial design with four types of plant communities and four earthworms/ herbivore treatments. We selected three plant species from three different plant functional groups and thus represent a broad range of functional characteristics, that were commercially available and observed in the native plant community from Canadian forest understories (Hallworth and Chinnappa 1997, Eisenhauer et al. 2007, Macdonald and Fenniak 2007, Royer and Dickinson 2007, Eisenhauer et al. 2009c), namely grass *Poa palustris*, legume *Vicia americana* and the herb *Symphyotrichum laeve*. They were each grown in monoculture (three seedlings of the same species) and in mixture (i.e. all three species together, with one seedling per species); resulting in four plant community treatments. We manipulated the presence of earthworms (*Lumbricus terrestris*, anecic species from Europe, but invasive across northern North America (Hendrix et al. 2006, 2008))

and aboveground herbivores (larvae of *Spodoptera exigua*, a chewing generalist herbivore with a worldwide distribution (Greenberg et al. 2001, Zheng et al. 2011) that is commonly used in laboratory experiments (Bezemer et al. 2003)) in a full-factorial design: 1) control (no earthworms, no herbivores), 2) with earthworms only, 3) with herbivores only and 4) with both earthworms and herbivores. Each of the 16 different treatment combinations (4× plant communities, 2× earthworms, 2× herbivores) was replicated six times, resulting in 96 microcosms. The experiment was conducted for 82 days. Microcosms were randomly distributed on four tables in an air temperature-controlled climate chamber with 60% humidity, at a day/night cycle of 16:00/8:00 h and 23/15 ± 0.9°C (mean ± SD), with a light intensity of approximately 400–450 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR). We simulated forest conditions of light availability which is highly variable in aspen forest depending on the forest patch location, and has previously been simulated to range between 300 and 900 µmol m^{-2} s^{-1} of PAR, using light of a wavelength between 400 and 700 nm (Eisenhauer et al. 2012, Thakur et al. 2021). Each microcosm was watered every 2–3 days during the experiment. The amount of water added (20–100 ml) depended on the overall dryness of the microcosms, but all microcosms always received the same amount of water at the same day. Microcosms were randomly redistributed between the tables every two weeks, but always at least one replicate of each treatment was placed on each table.

Microcosms consisted of PVC tubes (inner diameter 10 cm, height 24 cm) with a 700 µm mesh glued to the bottom to allow drainage of water. At the top of the tube, a barrier of transparent plastic film (height 15 cm) was attached to prevent earthworms from escaping (Eisenhauer et al. 2012). Soil (sandy-clay soil, pH = 7.38; N = 0.06%; C = 1.20%; C:N = 20.98) was ordered from a commercial supplier, and each microcosm was filled with 3 kg of sterile (i.e. autoclaved at 120°; 90 min) soil. We acknowledge that the pH and nutrients of the used soil differed from those of North American forest soils, but they were kept similar between all microcosms in order to disentangle earthworm effect on soil properties. To leach nutrients and compounds released during the sterilization procedure, 1 l of water was added to each microcosm.

Then, a soil microbial inoculation, using native soil that was sampled in September 2020 in an uninvaded aspen forest of the Kananaskis Valley (Alberta, Canada), was performed by directly mixing soil and by using a microbial wash. Native soil was shipped frozen, stored at −20°C until usage and, after defrosting, sieved through a 4 mm mesh. For each microcosm, we homogenized the 3 kg of sterilized soil with around 137 g of the Canadian soil in a sterilized (70% ethanol) bucket, before filling it back to the microcosm. To perform the microbial wash, we mixed 46 g of Canadian soil with 100 ml of water and poured it into each microcosm, followed by another 100 ml of water to dissolve the remains of the Canadian soil from the vessel. To ensure microbial community development, we stored the microcosms for ten days at

room temperature (20–25°C) and held their soil water content at 40–60% of their maximum water-holding capacity.

Plant material

Seeds were sown in soil from the Bad Lauchstädt Experimental Research Station of the Helmholtz Centre for Environmental Research- UFZ (11°53′E 51°24′N). Prior to sowing, the soil was autoclaved (120°C; 90 min), washed with tap water and inoculated using both approaches described above. Seeds of *V. americana* were scarified using sandpaper (grit size 150) and sown at 3–6 mm soil depth, *S. laeve* at 2 mm soil depth and *P. palustris* at the soil surface. Seeds then germinated in a reach-in climate chamber (night temperature: 18°C, day temperature: 22°C, duration day/night: 16:00/8:00 h; light intensity day: 450 µmol m^{-2} s^{-1} (partial light at 85%), humidity: 70%). After six weeks, the seedlings were transplanted into microcosms, and their vegetative height was measured (mean ± SD: *P. palustris*: 11.5 ± 3.1 cm; *S. laeve*: 0.7 ± 0.3 cm; *V. americana*: 8.8 ± 2.3 cm). Note the initial height differences among plant species due to the specificity of each plant functional group; the plant individuals were randomly distributed for the treatments. We observed a significant initial difference of 0.11 cm between *S. laeve* individuals that received no herbivores (0.70 ± 0.25 cm) and *S. laeve* individuals that received herbivores (0.59 ± 0.27 cm) (Supporting information). Further, *V. americana* individuals growing in mixture not receiving herbivores (9.58 ± 2.47 cm) were initially 2.58 cm taller by chance than *V. americana* individuals in mixture that received herbivores (7.00 ± 2.08 cm) (Supporting information). We accounted for these initial height differences in the statistical analyses by using the average initial height as covariate in the models. In each microcosm, the respective three plants were planted in a triangle approximately 5 cm from each other.

Earthworm treatment

Earthworms were ordered from a commercial supplier and handled according to Fründ et al. (2010). We sorted them into juveniles and adults, according to the presence or absence of a clitellum, and weighed them (after starving for 24 h at 12°C). The body mass of adults ranged from 2.8 to 4.6 g (fresh weight of living earthworms; mean ± SD: 3.6 ± 0.5 g), while the weight of juveniles ranged from 1.8 to 3.5 g (2.6 ± 0.4 g). Pairs of adults and juveniles were formed to add a similar total body mass of earthworms per microcosm (mean ± SD: 6.2 ± 0.12 g). Earthworms were added to half of the microcosms (with earthworm treatment). Four grams of autoclaved (120°C; 90 min) and cut (~ 1 cm²) litter of poplar species (*Populus* sp.; C:N = 22.6) sampled in a forest close to Leipzig, was added to each microcosm to feed earthworms and simulate litter conditions in uninvaded aspen forests.

Herbivore treatment

Herbivores were added in the last week of the experiment and left on the plants for five days in clip cages to prevent them from escaping. Prior to their addition, herbivores were starved for one day and weighed. One clip cage was added to the second (or third if needed) green and healthy leaf per plant, fixing ~ 50% of it in the cage, and receiving one herbivore each. In total, 141 herbivores were added (three plant individuals were dead at this point in time) to half of the microcosms (with herbivore treatment), and plants without herbivore treatment also received an empty clip cage to control for potential side effects. Clip cages consisted of two foam rings of pipe insulation (inner diameter: ~ 3 cm, thickness: ~ 1.5 cm) with mesh of 250 µm closing it on both sides, held together by three hair clips and fixed by a wire to a wooden stick next to the plant. After herbivore removal, the area consumed by the larvae was visually estimated according to predefined classes (0, < 1, 1–5, 6–15, 16–25, 26–50, 51–75 and > 76%). The median values of these classes (0, 0.5, 3, 10, 20, 38, 63 and 88) were used for further analyses. No herbivores were found in the control treatments.

Plant trait measurements

Fourteen plant functional traits were measured on the three plant species. They were selected as they reflect above- and belowground plant development, also the plant strategy in terms of resource acquisition, competition, reproduction, and response to disturbance and herbivory. Aboveground, we measured the individual biomass that is correlated to the overall plant performance; leaf area and the specific leaf area (SLA) that are correlated to the growth rate and resource acquisition; LDMC and leaf carbon content to evaluate plant investment in structural tissue and defenses; and leaf nitrogen content (and C:N ratio) to estimate plant nutritional quality. We further considered height as a plant functional trait (Cornelissen et al. 2003, Pérez-Harguindeguy et al. 2013) related to resource acquisition and competitive abilities. Belowground, we estimated plant resource uptake availability by calculating the specific root length (SRL), plant performance with the root tissue density (RTD) which is correlated to the growth rate, as well as their investment in belowground tissue via the root average diameter (mm) and root dry matter content (RDMC) (Cornelissen et al. 2003, Pérez-Harguindeguy et al. 2013, Kramer-Walter et al. 2016). The number of ramets and inflorescences were measured to obtain information about the reproductive abilities of the plants.

Aboveground plant traits were measured for each plant individual. After herbivory rate estimation, ramets were counted, and the height was measured (for *S. laeve* and *P. palustris*, we measured the vegetative height; for *V. americana*, we measured the stretched height due to its growth form). Further analyses were conducted with the height growth values to exclude initial height differences among plant individuals and treatments. As it was not possible to distinguish the different individuals in the monoculture treatment at the end of the experiment, we calculated the height growth of plants in monoculture, by subtracting the mean initial height per microcosm from the final height of each plant individual. For mixtures, the respective initial height was subtracted from

the final height of each individual plant. In microcosms with plant mixtures, inflorescences (only *P. palustris* developed inflorescences) were documented per plant individual; while in monocultures, they were counted per microcosm, divided by three, and this average value was then used once per (grass) monoculture microcosm for further analysis. Three green and healthy leaves including the petiole (preferably the 3rd, 4th and 5th youngest) that did not receive herbivores before, were cut off the plant, weighed and scanned fresh using a CanoScan LIDE220 scanner at 600 dpi in grayscale. The leaf that was secured in the clip cage (irrespective of herbivore treatment) was previously taken for metabolomic measurements, which are not part of this manuscript. The aboveground biomass was then cut off and stored in paper bags.

To measure belowground plant traits, the soil was removed from the microcosms. Earthworms were collected, starved again and reweighed after 24 h. Soil samples for pH and water content (each 30 g) analyses were taken and stored at − 20°C until further processing. Roots from each plant individual were disentangled and thoroughly washed with water. Debris and black/dead parts of roots were picked out using tweezers. Roots were sorted into fine roots (< 2 mm diameter) and coarse roots (> 2 mm diameter). Detached roots were grouped as community roots (including small root pieces from the soil), and the water used for cleaning the roots was sieved (250 µm) to avoid loss of any root material. Further analyses of belowground traits were done on one random individual from plant monocultures and all individuals from plant mixtures. Depending on the mass of the fine roots, either the whole sample or a representative fraction (mean ± SD: 0.6 ± 0.6 g) of it was weighed and scanned using an Epson Perfection 11000XL scanner at 600 dpi in grayscale.

All samples (aboveground biomass, scanned leaves and all root samples) were oven-dried at 60°C for 72 h and weighed again to assess their dry mass. Above- and belowground community (microcosm) biomasses were determined by adding the respective dry weights, and the community shoot:root ratio was calculated.

Leaf scans were analyzed using the software WinFOLIA (ver. 2014a Pro). Leaf area (cm^2) was provided by the software, and we calculated SLA (leaf area divided by its dry mass (mm^2 mg^{-1})), and LDMC (dry mass divided by fresh mass (mg g^{-1})) using standardized protocols (Pérez-Harguindeguy et al. 2013). For SLA and LDMC, an average per individual was determined. Root scans were analyzed using the software WinRhizo (ver. 2013e Pro), removing objects with an area smaller than 0.001 cm^2. Larger debris pieces, air bubbles and scratches were manually excluded. Root average diameter (mm) was provided by the software, and we calculated specific root length (SRL; root length divided by dry mass (m g^{-1})), RTD (dry mass divided by volume (g cm^{-3})) and RDMC (dry mass divided by fresh mass (mg g^{-1})) (Pérez-Harguindeguy et al. 2013, Roumet et al. 2016). For carbon (C) and nitrogen (N) content measurement, leaves of the same plant individual were pooled, and 30 g of soil of each microcosm was analyzed. All samples

were dried (60°C for 72 h), ground and transferred into tin capsules (30 mg, respectively). Analysis was performed using dry combustion with a Vario EL cube IR elemental analyzer. C and N content were provided as the relative mass proportion of the element (%) per sample mass, and C:N ratio was calculated from these.

Measurements of soil abiotic properties

For the first four weeks of the experiment, one plastic strip with respectively one ion-exchange membrane glued to each side (IEMs, 2.5 × 2.5 cm; AMI-7001S and CMI-7000S) was added to each microcosm at 10 cm depth to assess soil inorganic N availability (Durán et al. 2013). Prior to addition, the membranes were immersed in 5% NaCl solution for 2 h at 37°C to allow for membrane hydration and expansion. After 29 days, the anion and cation membranes from IEMs were removed from the soil, air dried, brushed to remove soil particles and carefully removed from plastic strips. The extraction was performed with 2 M KCL by orbital spinning (1 h at 200 rpm). The extracts were analyzed for ammonium (NH_4^+), nitrate (NO_3^-) by the indophenol blue method (Sims et al. 1995) and phosphate (PO_4^{3-}) by the molybdenum blue method (Allen et al. 1986), with a microplate reader (Durán et al. 2008). One sample was excluded from statistical analysis, as we only found the plastic strip at the end of the experiment and not after 29 days (microcosm Grass-Control-2). For pH measurements, 10 g of air-dried soil was solved in 25 ml of 0.01 M $CaCl_2$ solution, shaken and left for 1 h. Measurements were conducted with a pH meter according to the manual. To determine soil water content, fresh soil was weighed, dried in an oven (75°C for at least 24 h) and reweighed.

Statistical analyses

Statistical analyses and figures were conducted and created, respectively, using the R software ver. 4.1.2 (www.r-project.org). To take into account the death of some herbivores, we subset the herbivore treatment prior to analyses: we only considered the plant individuals where we found 1) the herbivore alive and 2) trace(s) of leaf consumption, while the herbivore was dead, for our herbivore treatment (244 from 288 plant individuals were left for the analyses). At the community level, we removed all microcosms in which none of the three plant individuals showed traces of herbivory (microcosm-level traits; 93 from 96 microcosms left) prior to the analyses. Following this sub-setting, the treatment combination of mixture-earthworm-herbivore presence had a reduced dataset, with no individual trait data left for the leaf traits and nutrients (SLA, LDMC, leaf area and leaf nutrient) of *V. americana* and for the belowground traits (root average diameter, SRL, RDMC, RTD, shoot root ratio) of *S. laeve*. No other treatment combination was missing data compared to the dataset explained above. Each trait was analyzed using a linear model, followed by a type II ANOVA from the package 'car' (Fox and

Weisberg 2019) after removing missing data from dead plant individuals (2% NAs in the whole dataset, 1.5% NAs in the subsetted dataset). Plant species identity, plant diversity level, earthworm treatment and herbivore treatment were analyzed as factors in a four-way interaction, respectively. Assumptions of the models were visually checked using the R packages 'performance' (Lüdecke et al. 2021a) and 'see' (Lüdecke et al. 2021b). When needed, variables were log transformed, such as leaf area, SLA, RDMC, RTD, SRL, shoot:root ratio, C:N of leaves and soil, nitrogen content of leaves, PO_4^{3-} and NH_4^+ of soil. As the resin strips from which soil NO_3^-, PO_4^{3-} and NH_4^+ were determined, were in the soil before the herbivore treatment was applied, we only used plant species identity, plant diversity level and earthworm treatment as explanatory factors. To analyze height growth and aboveground biomass, the average initial height was added to the model as a covariate, but not in interaction with the other factors. For count (ramets) and proportion (survival data) data, generalized linear models (Poisson and binomial, respectively) were performed using the package 'stats' (www.r-project.org). As only *P. palustris* developed inflorescences, species identity was not considered for the analysis. We analyzed herbivore survival/rate and earthworm survival by excluding the respective treatments (i.e. herbivore or earthworm presence) from the explanatory variables of the model, and the control treatments (i.e. treatment without herbivore and/or earthworms) from the datasets. For community traits and soil abiotic measurements the species column included the necessary information (*S. laeve*, *P. palustris*, *V. americana* or mixture) and the plant diversity level was therefore excluded as an explanatory variable. Post hoc tests of the significant interactions were conducted using the function *emmeans* from the corresponding R package (Lenth 2021) with Holm correction and specifying 'weights=flat' to apply zero weight to missing factor combinations and thus receive marginal means despite missing data for certain combinations. Compact letter displays were created using the R package 'multcomp' (Hothorn et al. 2008). Data were plotted per trait using 'ggplot2' (Wickham 2016) and the letters correspond to the significant interactions including plant species identity. For calculating the change of traits in percentage, the estimated marginal means from the post hoc tests were used. Given that some earthworms died during the experiment, we performed sensitivity analyses by running the same analyses for our different variables on the datasets where we removed the data points where both earthworms had died (individual level: 237 from 288 left; microcosm level: 90 from 96 left). A comparison of the results between these two analyses (with and without dead earthworms) shows only minor changes for the belowground community productivity and soil C:N ratio, which are presented in the Results section. See the Supporting information for all results, including the sensitivity analyses. The following abbreviations are used for the statistical effects in the results: earthworm treatment=EW; herbivore treatment=Hrb; plant species identity=Spc; Plant diversity level=Clt.

Results

Plants, earthworms, herbivores survival and herbivory rate

Out of the 96 earthworms added to experimental microcosms, 68 were found alive at the end of the experiment (70%), which is comparable to other microcosm studies (Wurst et al. 2008, Eisenhauer et al. 2012). In our study, no experimental treatment influenced the earthworms' survival significantly (Supporting information), but the death of herbivores was significantly affected by the interaction between the plant species identity and plant diversity level (Clt:Spc effect: p=0.011, Supporting information). We found that 118 herbivores died or escaped (84%) after five days, while 97 out of 141 leaves (64%) had marks of herbivory on them. The highest proportion of herbivores survived on *S. laeve* growing in monoculture (44%) followed by *V. americana* monoculture (17%), while no herbivores survived on these plant species growing in mixture. One herbivore survived on *P. palustris* growing in mixture, none when growing in monoculture (Supporting information). Overall, the *S. exigua* larvae fed most on *S. laeve*, second on *V. americana* and the least on *P. palustris* (Spc effect: p=0.017; Supporting information). Earthworm presence had no significant effect on the rate of consumption by herbivores (herbivory rate). Only five out of the 288 plant individuals died during the experiment (1.7%), four of which were *V. americana* individuals growing in mixture and one *S. laeve* from a monoculture (Supporting information). In the dataset where plants without herbivore consumption and dead herbivores were removed, only one plant (*V. americana* from mixture) out of 244 died.

Effects of earthworms and plant community on soil abiotic properties

Soil pH (7.41 ± 0.05) was not significantly affected by the experimental treatments (Supporting information). Soil water content was significantly lower (− 10%) in the presence of earthworms (EW effect: p=0.023; Supporting information). Soil C content did not differ among plant species, but soil of *P. palustris* communities contained less C when earthworms were present (− 20%; EW:Spc interaction effect: p=0.011; Supporting information), and soil of *V. americana* communities had an increased soil C content in the presence of herbivores (+ 16%; Hrb:Spc interaction effect: p=0.014). Soil of *P. palustris* communities contained significantly more total N without earthworms and herbivores present (+ 35%) compared to other treatment combinations (EW:Spc:Hrb interaction effect: p=0.023; Supporting information).

In the absence of earthworms, soil C:N ratio was higher (+ 17%) when herbivores were present (EW:Hrb interaction effect: p=0.035; Supporting information). This interaction was further modulated by species identity (EW:Spc:Hrb interaction effect: p=0.035; Supporting information) when performing the sensitivity analysis: we now only found that *P. palustris* monocultures (+ 55%) and soil of *S. laeve*

monocultures had a higher C:N ratio when herbivore and earthworms were present (+ 35%) compared to when herbivore and earthworms were absent. No significant effect for *V. americana* monocultures or mixtures was found.

Within the first four weeks of the experiment, no other factor apart from plant species identity had a significant effect on phosphate PO_4^{3-} (Spc effect: p = 0.0003; Supporting information). In the presence of earthworms, soil nitrate NO_3^- increased significantly (+ 54%; EW effect: p = 0.0002; Supporting information), while ammonium NH_4^+ did not change significantly in response to any treatment, including plant species identity and earthworm treatments (Supporting information).

Plant community productivity responses

In the presence of earthworms, *P. palustris* and mixed communities had a significantly higher community aboveground productivity than when earthworms were absent (*P. palustris*: + 27%, mixed community: + 24%; EW:Spc interaction effect: p = 0.0004; Fig. 1, Supporting information), while there were no significant changes for *S. laeve* and *V. americana*.
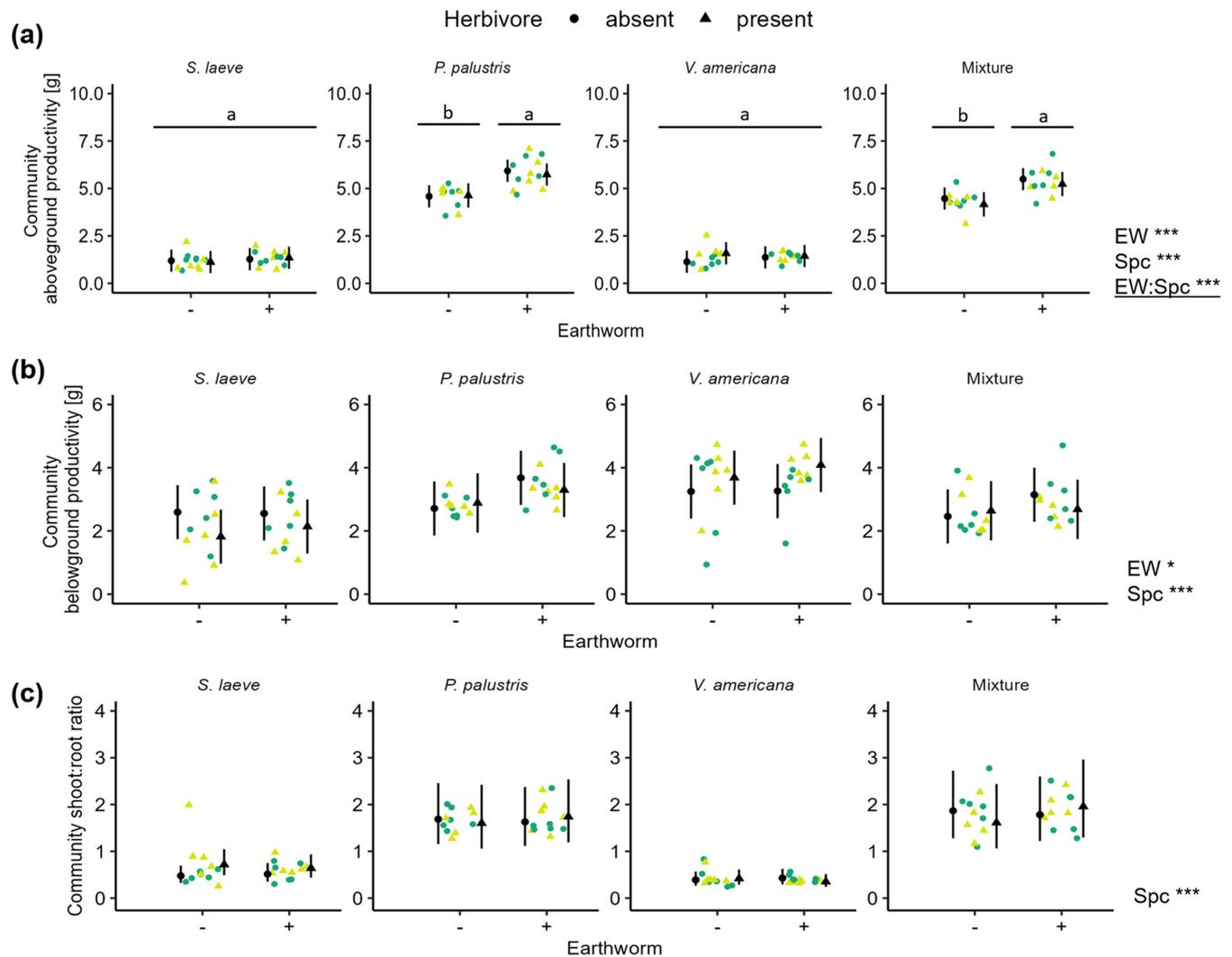
Figure 1. Community aboveground productivity (a), community belowground productivity (b) and community shoot:root ratio (c) (all per microcosm) (estimated marginal mean ± 95% CI) of different plant communities (monocultures of *Symphyotrichum laeve*, *Poa palustris* and *Vicia americana*, or a mixture of all three species) with (+) and without (−) earthworms as well as herbivore treatment (presence (yellow triangles) versus absence (green circles)). A linear model was applied to analyze these data (community shoot:root ratio was log transformed, data were back transformed before plotting). Significant factors are displayed on the right side next to the figure, respectively (significance levels: '***' 0.001; '**' 0.01; '*' 0.05). The letters were derived from a post hoc test (pairwise comparison of estimated marginal means using the 'holm' correction) investigating the significant interaction (underlined in the statistics legend). Different letters represent a significant difference among means (p value < 0.05) and can be interpreted only within each community. 93 observations were analyzed (*S. laeve*: 24; *P. palustris*: 23; *V. americana*: 24; mixture: 22; earthworm absence/presence: 46/47; herbivore absence/presence: 48/45). EW: earthworm treatment; Spc: plant species identity.

Root community productivity was overall higher (+ 13%) when earthworms were present (EW effect: p = 0.039, Fig. 1, Supporting information). This effect was only marginally significant (EW effect: p = 0.064; Supporting information) in the sensitivity analysis. Community shoot:root ratio was not significantly affected by earthworm presence (Fig. 1, Supporting information). Herbivore treatment did not affect any of the community variables alone or in interaction (Supporting information).

Plant functional trait responses

All studied plant functional traits differed significantly among plant species. Eight out of fourteen plant functional traits were significantly affected by earthworm presence, either by a general effect across all treatments, or the earthworm effect differed significantly among plant species, plant diversity level and/or herbivore presence.

Aboveground trait responses

Among the aboveground traits measured, earthworm treatment overall affected individual aboveground biomass, SLA and LDMC, but not the leaf area and height growth (Supporting information). Earthworm presence increased aboveground biomass of *P. palustris* individuals (+ 20%; EW:Spc interaction effect: p < 0.0001), but not of the other two species (Fig. 2). Individual aboveground biomass also changed according to the plant diversity level: *S. laeve* and *V. americana* grew less aboveground biomass when growing in a mixture (*S. laeve*: − 93%; *V. americana*: − 89%),
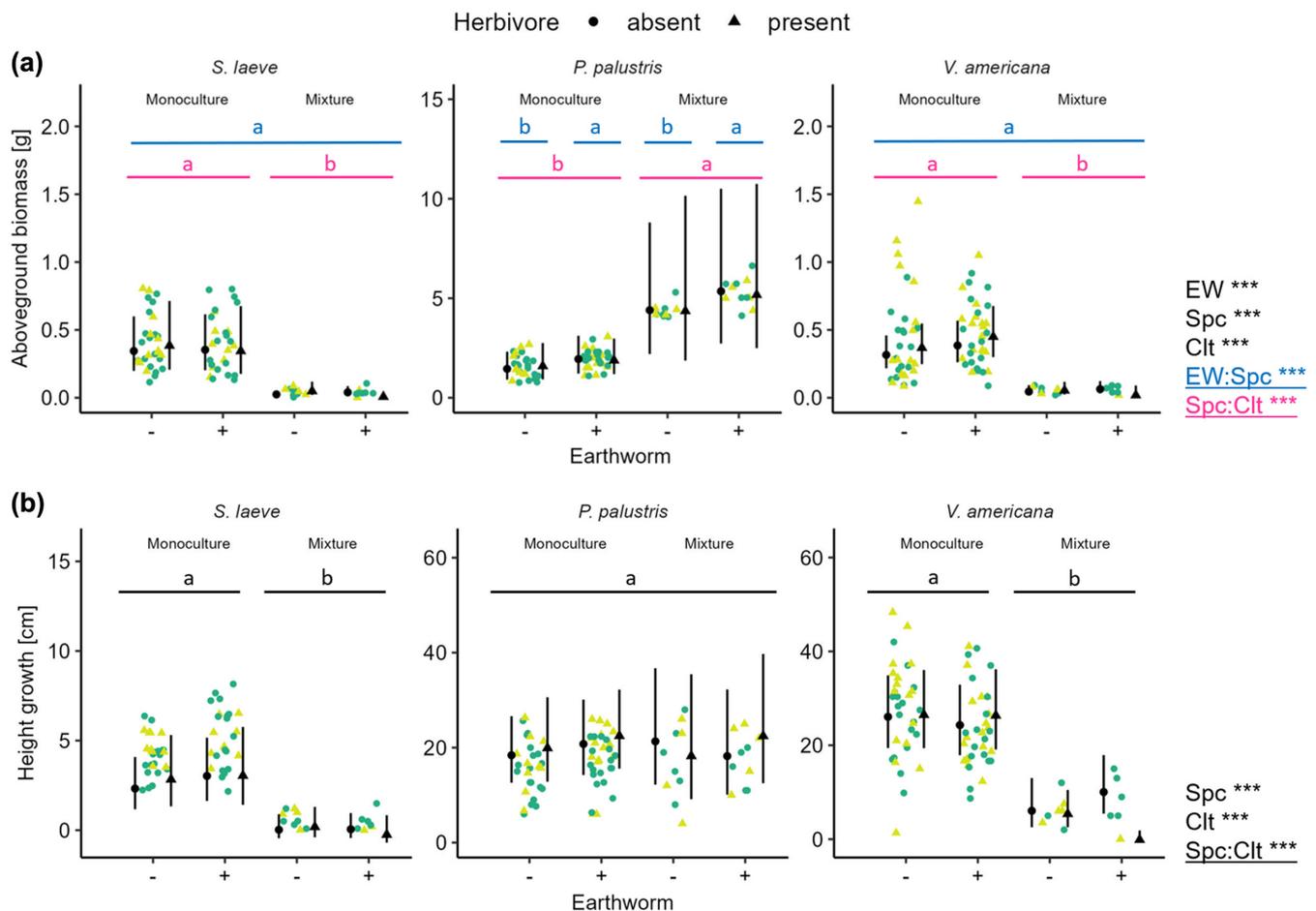
Figure 2. Individual aboveground biomass (a) and height growth (b) (estimated marginal mean ± 95% CI) of the three plant species studied (*Symphyotrichum laeve*, *Poa palustris* and *Vicia americana*) growing in different plant diversity levels (monoculture versus mixture) with (+) and without (−) earthworms as well as herbivore treatment (presence (yellow triangles) versus absence (green circles)). A linear model was applied to analyze these data. Significant factors are displayed on the right side next to the figure, respectively (significance levels: '***' 0.001; '**' 0.01; '*' 0.05). The letters were derived from a post hoc test (pairwise comparison of estimated marginal means using the 'holm' correction) investigating the significant interactions (underlined in the statistics legend). Different letters represent a significant difference (p value < 0.05) and can be interpreted only within each community. (a) 243 observations were analyzed (*S. laeve*: 75; *P. palustris*: 83; *V. americana*: 85; monoculture/mixture: 188/55; earthworm absence/presence: 121/122; herbivore absence/presence: 143/100). (b) 235 observations were analyzed (*S. laeve*: 71; *P. palustris*: 82; *V. americana*: 82; monoculture/mixture: 186/49; earthworm absence/presence: 116/119; herbivore absence/presence: 137/98). EW: earthworm treatment; Spc: plant species identity; Clt: plant diversity level.
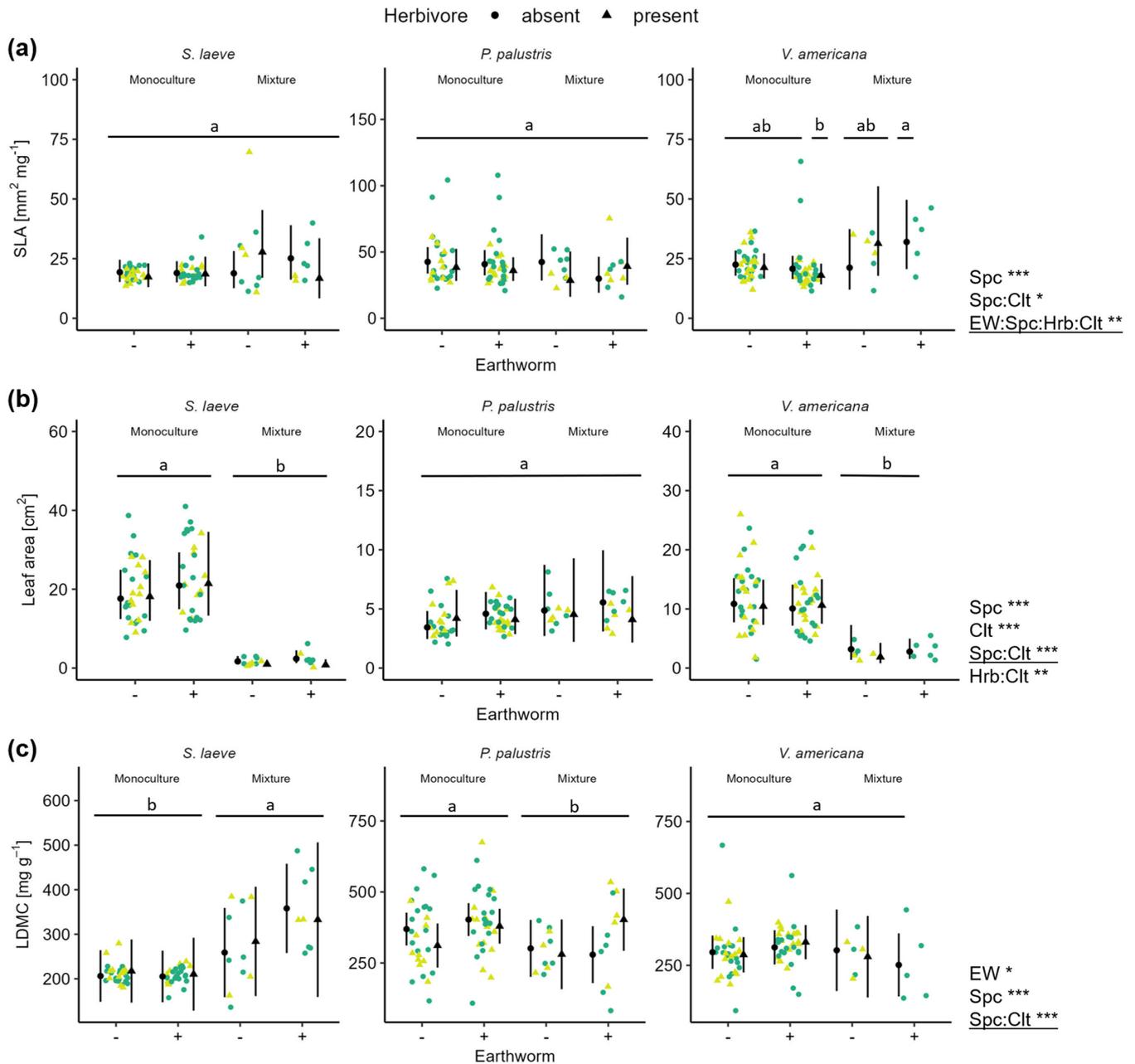
Figure 3. Specific leaf area (a), leaf area (b) and leaf dry matter content (c) (estimated marginal mean ± 95% CI) of the three plant species studied (*Symphyotrichum laeve*, *Poa palustris* and *Vicia americana*) growing in different plant diversity levels (monoculture versus mixture) with (+) and without (−) earthworms as well as herbivore treatment (presence (yellow triangles) versus absence (green circles)). A linear model was applied to analyze these data (leaf area and SLA were log transformed, data were back transformed before plotting). Significant factors are displayed on the right side next to the figure, respectively (significance levels: '***' 0.001; '**' 0.01; '*' 0.05). The letters were derived from a post hoc test (pairwise comparison of estimated marginal means using the 'holm' correction) investigating significant interactions (underlined in the statistics legend). Different letters represent a significant difference (p value < 0.05) and can be interpreted only within each community. (a) 234 observations were analyzed (*S. laeve*: 73; *P. palustris*: 81; *V. americana*: 80; monoculture/mixture: 187/47; earthworm absence/presence: 116/118; herbivore absence/presence: 137/97). (b) 237 observations were analyzed (*S. laeve*: 73; *P. palustris*: 83; *V. americana*: 81; monoculture/mixture: 187/50; earthworm absence/presence: 117/120; herbivore absence/presence: 139/98). (c) 237 observations were analyzed (*S. laeve*: 75; *P. palustris*: 83; *V. americana*: 79; monoculture/mixture: 187/50; earthworm absence/presence: 118/119; herbivore absence/presence: 139/98). EW: earthworm treatment; Spc: plant species identity; Clt: plant diversity level; Hrb: herbivore treatment.
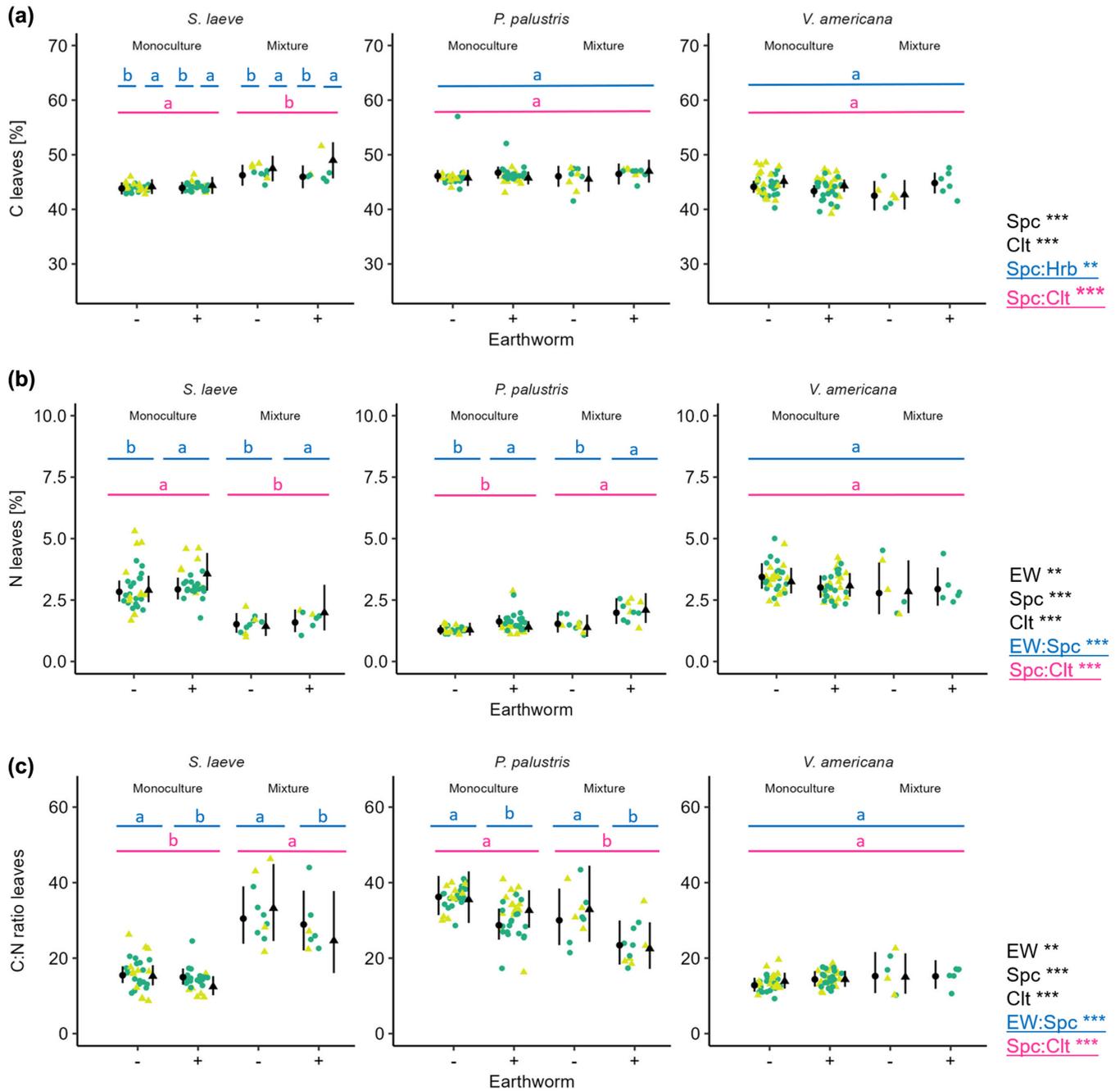
Figure 4. Leaf carbon content (a), leaf nitrogen content (b) and leaf C:N ratio (c) (estimated marginal mean ± 95% CI) of the three plant species studied (*Symphyotrichum laeve*, *Poa palustris* and *Vicia americana*) growing in different plant diversity levels (monoculture versus mixture) with (+) and without (−) earthworms as well as herbivore treatment (presence (yellow triangles) versus absence (green circles)). A linear model was applied to analyze these data (leaf nitrogen and C:N ratio log transformed, data were back transformed before plotting). Significant factors are displayed on the right side next to the figures (significance levels: '***' 0.001; '**' 0.01; '*' 0.05). The letters were derived from a post hoc test (pairwise comparison of estimated marginal means using the 'holm' correction) investigating the significant interactions (underlined in the statistics legend). Different letters represent a significant difference (p value < 0.05) and can be interpreted only within each community. 238 observations were analyzed (*S. laeve*: 74; *P. palustris*: 83; *V. americana*: 81; monoculture/mixture: 188/50; earthworm absence/presence: 118/120; herbivore absence/presence: 140/98). EW: earthworm treatment; Spc: plant species identity; Clt: plant diversity level.
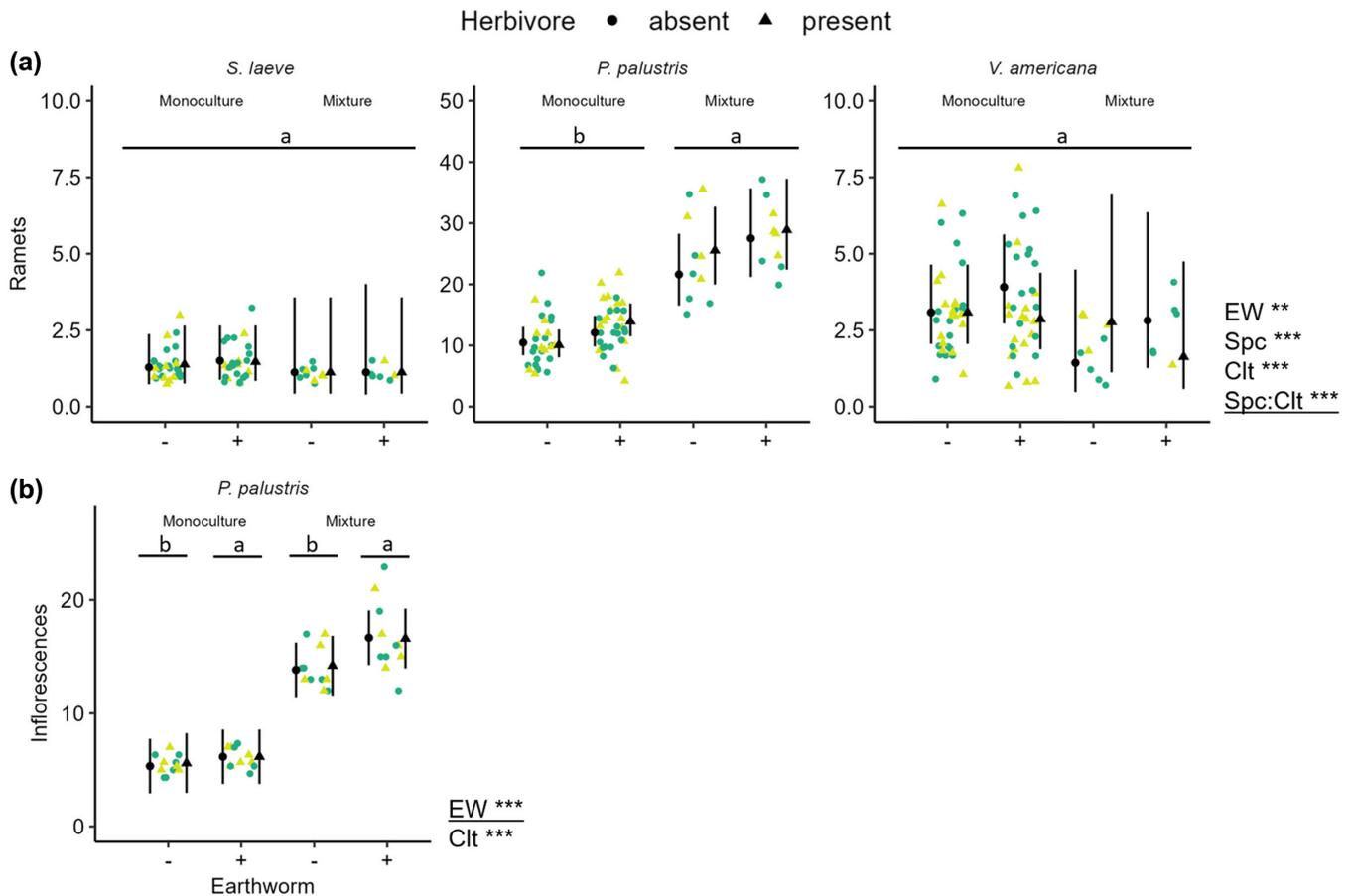
Figure 5. Ramets (a) (estimated marginal mean ± 95% CI) of the three plant species studied (*Symphyotrichum laeve*, *Poa palustris* and *Vicia americana*) and inflorescences of *P. palustris*; and (b) (estimated marginal mean ± 95% CI) growing in different plant diversity levels (monoculture versus mixture) with (+) and without (−) earthworms as well as herbivore treatment (presence (yellow triangles) versus absence (green circles)). A generalized linear model (family = Poisson (link = 'log')) was applied to analyze ramets (data were back transformed before plotting) and a linear model was applied to analyze inflorescences. Significant factors are displayed on the right side next to the figures, respectively (significance levels: '***' 0.001; '**' 0.01; '*' 0.05). The letters were derived from a post hoc test (pairwise comparison of estimated marginal means using the 'holm' correction) investigating the significant factor/interaction (underlined in the statistics legend). Different letters represent a significant difference (p value < 0.05) and can be interpreted only within each community. (a) 237 observations were analyzed (*S. laeve*: 72; *P. palustris*: 81; *V. americana*: 84; monoculture/mixture: 186/51; earthworm absence/presence: 119/118; herbivore absence/presence: 140/97). (b) 83 observations were analyzed (all *P. palustris*; monoculture/mixture: 62/21; earthworm absence/presence: 38/45; herbivore absence/presence: 48/35). EW: earthworm treatment; Spc: plant species identity; Clt: plant diversity level.

while *P. palustris* produced more aboveground biomass when growing in a mixture (+ 182%; Spc:Clt interaction effect: p < 0.0001). Overall, *V. americana* and *P. palustris* individuals had the largest height difference between initial and final height (*V. americana*: 22.50 ± 11.09 cm, *P. palustris*: 16.91 ± 5.75 cm), while *S. laeve* individuals grew the least (3.72 ± 2.13 cm; Fig. 2). Earthworm and herbivore treatments did not significantly affect the height growth of any plant species (Fig. 2).

In the presence of earthworms, the SLA of *V. americana* was higher in the absence of herbivores in a mixture (+ 20%) in comparison to the treatment with herbivores in monoculture, while the other treatment combinations had a similar SLA (EW:Hrb:Clt:Spc interaction effect: p = 0.002; Fig. 3, Supporting information). Leaf area was not significantly affected by earthworm presence, but *S. laeve* and *V.*

americana had lower leaf areas when growing in a mixture (*S. laeve*: − 90%; *V. americana*: − 60%; Spc:Clt interaction effect: p < 0.0001; Fig. 3, Supporting information). Further, across all species, individuals growing in a mixture had the lowest leaf area when herbivores were present (− 42%, Hrb:Clt interaction effect: p = 0.004; Supporting information). The LDMC was generally higher (+ 11%) when earthworms were present (EW effect: p = 0.015; Fig. 3, Supporting information).

Earthworms affected the N content and C:N ratio of the leaves (Supporting information). The C content of leaves was not affected by the earthworm treatment, but by the herbivore treatment: leaves of *S. laeve* individuals had more C when herbivores were present (+ 3%; Hrb:Spc interaction effect: p = 0.007; Fig. 4, Supporting information). The leaf N content of *P. palustris* (+ 80%) *and S. laeve* (+ 21%) was
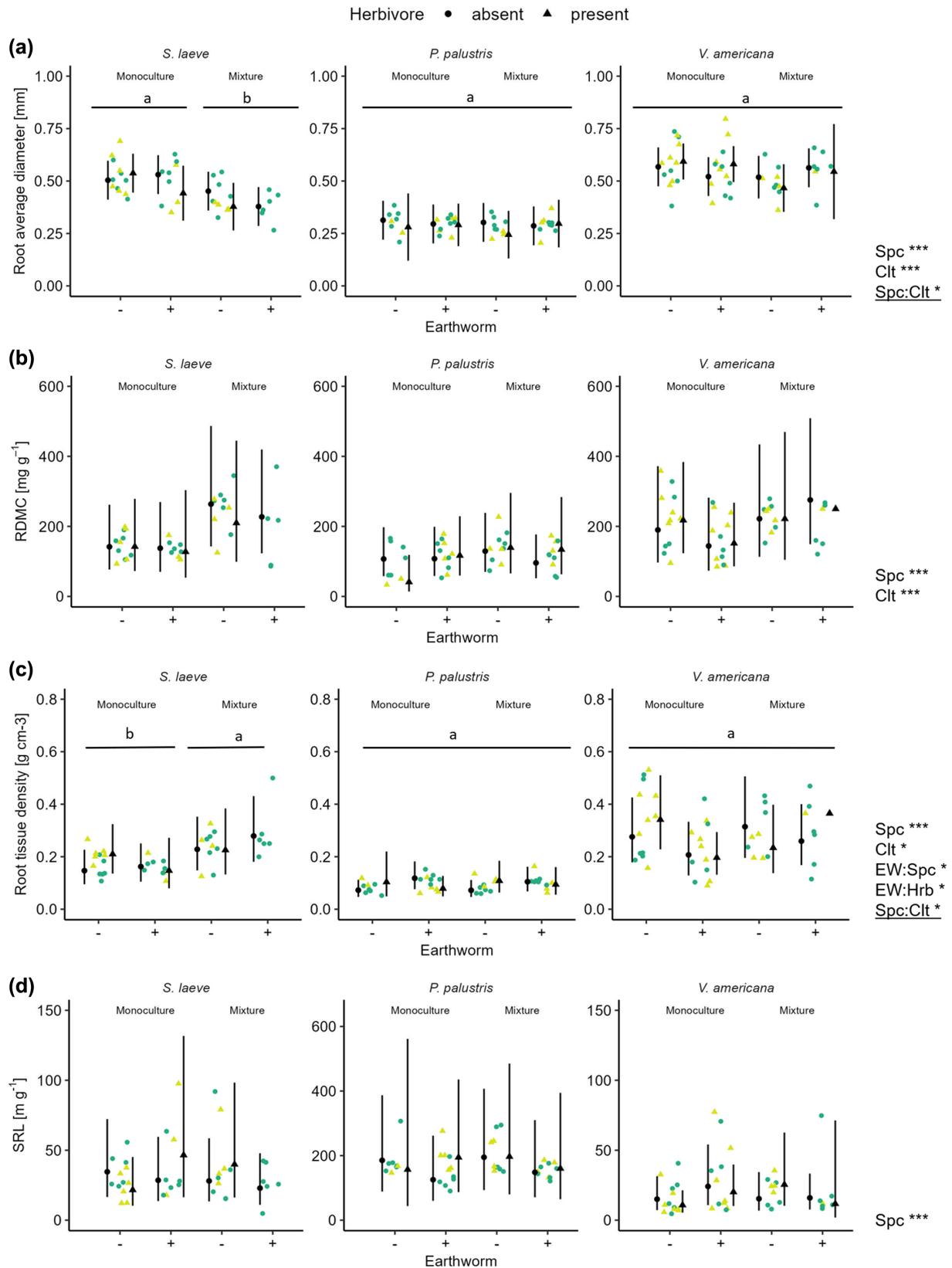
Figure 6. Root average diameter (a), root dry matter content (b), root tissue density (c) and specific root length (d) (estimated marginal mean ± 95% CI) of the three plant species studied (*Symphyotrichum laeve*, *Poa palustris* and *Vicia americana*) growing in different plant diversity levels (monoculture versus mixture) with (+) and without (−) earthworms as well as herbivore treatment (presence (yellow triangles) versus absence (green circles)). A linear model was applied to analyze these data (root tissue density and SRL were log transformed, data were back

Figure 6. Continued.

transformed before plotting). Significant factors are displayed on the right side next to the figure, respectively (significance levels: '***' 0.001; '**' 0.01; '*' 0.05). The letters were derived from a post hoc test (pairwise comparison of estimated marginal means using the 'holm' correction) investigating the significant interactions (underlined in the statistics legend). Different letters represent a significant difference (p value < 0.05) and can be interpreted only within each community. Despite significant results for the interactions EW:Spc and EW:Hrb of the linear model testing RTD, the post hoc test did not indicate significant differences among means. (a) 118 observations were analyzed (*S. laeve*: 37; *P. palustris*: 39; *V. americana*: 42; monoculture/mixture: 66/52; earthworm absence/presence: 62/56; herbivore absence/presence: 71/47). (b) 114 observations were analyzed (*S. laeve*: 35; *P. palustris*: 39; *V. americana*: 40; monoculture/mixture: 62/52; earthworm absence/presence: 60/54; herbivore absence/presence: 68/46). (c) 117 observations were analyzed (*S. laeve*: 37; *P. palustris*: 39; *V. americana*: 41; monoculture/mixture: 65/52; earthworm absence/presence: 62/55; herbivore absence/presence: 70/47). (d) 117 observations were analyzed (*S. laeve*: 37; *P. palustris*: 39; *V. americana*: 41; monoculture/mixture: 65/52; earthworm absence/presence: 62/55; herbivore absence/presence: 70/47). EW: earthworm treatment; Spc: plant species identity; Clt: plant diversity level; Hrb: herbivore treatment.

increased in the presence of earthworms (EW:Spc interaction effect: p = 0.0004; Fig. 4) and both species had a lower C:N ratio with earthworms being present (*S. laeve*: − 5%; *P. palustris*: − 7%; EW:Spc interaction effect: p = 0.004), while no significant difference was found for *V. americana* (Fig. 4, Supporting information).

Reproductive trait responses

While only *P. palustris* individuals developed inflorescences that were produced in higher numbers in a mixture (+ 163%; Clt effect: p < 0.0001) and when earthworms were present (+ 17%; EW effect: p = 0.010; Fig. 5, Supporting information), vegetative reproductive abilities (i.e. number of ramets) were overall increased for all species in the presence of earthworms (+ 25%; EW effect: p = 0.001; Fig. 5, Supporting information) and *P. palustris* produced generally more ramets in plant mixtures (+ 124% Spc:Clt interaction effect: p < 0.0001; Fig. 5). Herbivore treatment alone or in interaction did not affect plant reproductive traits (Supporting information).

Belowground trait responses

We found coarse roots for *V. americana* (46 samples) and *S. laeve* (four samples), while *P. palustris* only had fine roots. Only RTD measured on the fine root samples varied in response to the earthworm treatment and the herbivore treatment (Fig. 6, Supporting information). The roots of *S. laeve* had a higher RTD when growing in a mixture (+ 47%; Spc:Clt interaction effect p = 0.011; Fig. 6), while the other species were not affected. RTD was also affected by the interactions EW:Spc and EW:Hrb, whereas no significant pairwise differences were detected in the post hoc tests. However, EW:Spc:Hrb interaction tended to be significant in the linear model (p = 0.086) and pairwise comparison showed that, in the absence of herbivores, *P. palustris* has a higher RTD when earthworms were present (+ 54%) compared to when earthworms were absent. RDMC, root average diameter and specific root length (SRL) were not significantly affected by the earthworm or herbivore treatment. RDMC was higher in a mixture compared to monoculture (+ 63%; Clt effect: p = 0.0004), while root average diameter of *S. laeve* was lower (− 20%, Spc:Clt interaction effect: p = 0.041) when growing in a mixture, and SRL only differed between species in general (Fig. 6, Supporting information).

Discussion

Earthworms change plant trait expression

Given that earthworm invasion is changing plant communities in North American forests towards lower diversity and dominance of grasses (Craven et al. 2017), we investigated the underlying mechanisms by examining how earthworms affect the functional traits of plant species belonging to three different plant functional groups (i.e. grass, herb and legume) in different plant diversity levels (i.e. different levels of competition: monoculture versus mixture) and in the presence and absence of aboveground herbivores. Our study confirms that invasive earthworms can affect plant functional traits of native plants in a significant way, which is in line with the recent findings of Blume-Werry et al. (2020) and Thouvenot et al. (2021). We observed that eight out of fourteen measured plant functional traits were affected either by the main effect of earthworms (i.e. LDMC, ramets and inflorescence production), or earthworm effects in interaction (i.e. individual aboveground biomass, SLA, leaf nitrogen content, leaf C:N ratio and RTD) with those of plant species identity, plant diversity level and/or herbivore presence (Fig. 7).

We did not observe a significant response to earthworm presence from the height growth, leaf area and leaf carbon content (only marginally), RDMC (only marginally), root average diameter and the specific root length. The absence of change in these plant traits could be explained by the investment of the plant resources towards other traits that we did not study such as the leaf thickness, the production of lignin or polyphenols that could also be investigated in this context in the future. Another explanation would be a too short duration of the experiment: some traits might need a longer time to change according to our treatment. For example, Cameron et al. (2014) found that plant roots occurred differently in soil cracks or earthworm burrows over time, given the species identity. Time also plays a role in the inflorescence production with the two species *S. laeve* and *V. americana* that did not produce flowers during our experiment while *P. palustris* did. This also shows that long-term effects of earthworms on plant traits should also be investigated in further studies. As seed production is often referred to as 'the fitness currency' of plants (Schwachtje et al. 2006) and flowering is
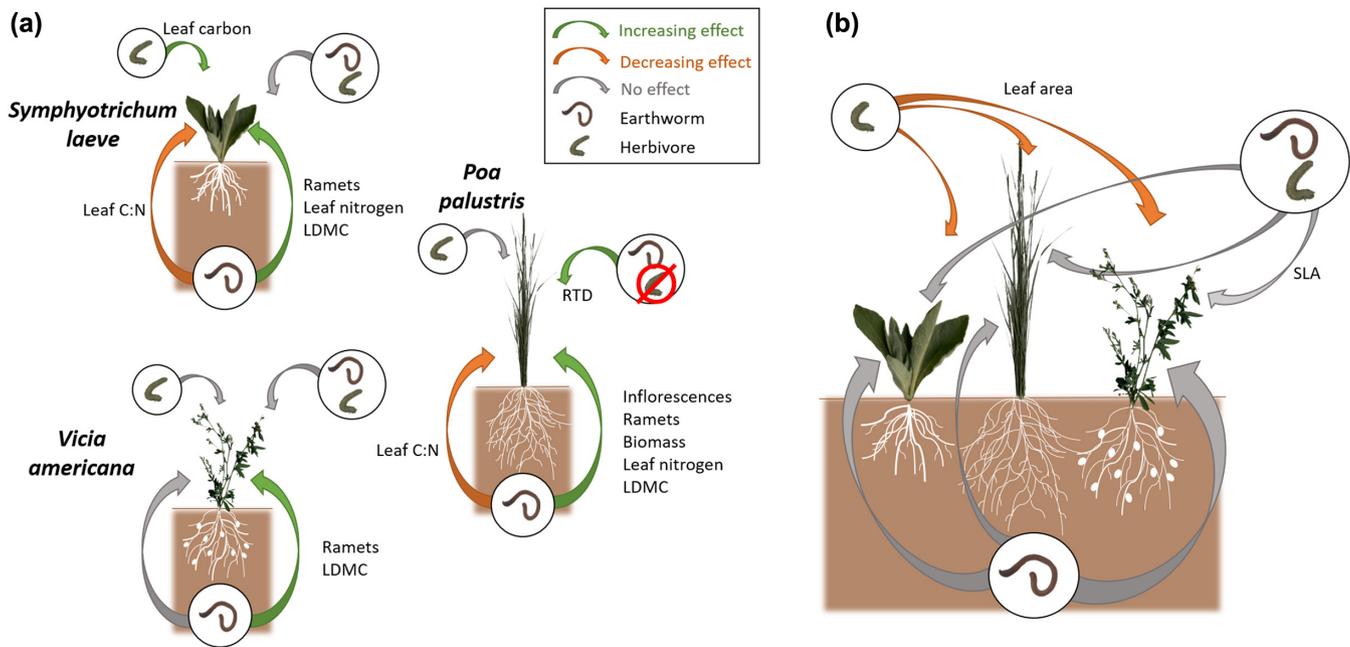
Figure 7. Direction of effects of treatments (earthworm and herbivore treatment per plant species) on plant functional traits of *Symphyotrichum laeve*, *Poa palustris* and *Vicia americana* (a). Modulation of plant functional traits of the three species by biotic interaction (b): earthworm, interspecific competition and herbivory. Plant diversity level is excluded from panel (a) and thus results are valid per species irrespective if the plant individuals grew in monoculture or in a mixture. Only changes that involve plant diversity level as a significant factor are shown in panel (b). LDMC: leaf dry matter content; SLA: specific leaf area.

a critical prerequisite, the effects of earthworms on flowering and seed production deserve more attention, and experiments carried out over a longer period of time are needed. Moreover, such studies would help in finding out whether the grasses only developed inflorescences earlier or more overall when earthworms were present.

However, earthworm treatment (alone) increased the number of ramets, inflorescences and the LDMC of plant individuals across all plant species. By modulating these traits, earthworms can stimulate plant reproduction/propagation and increase plant resistance to herbivory (Elger and Willby 2003). These changes might be based on the higher soil nutrient availability due to the activities of earthworms (Van Groenigen et al. 2014). Indeed, it was shown that LDMC is a predictor for soil fertility (Hodgson et al. 2011) and Zaller and Arnone (1999) explained increased ramet production close to earthworm casts mainly by enhanced nutrient availability. This is in line with increased concentrations of nitrate in soil and the higher leaf nutritional value in *S. laeve* and *P. palustris* (lower C:N and higher N, respectively) in the presence of earthworms. The observed increase of soil nitrate in the presence of earthworms supports the assumption that earthworms enhance soil nitrogen availability and uptake by plants (Van Groenigen et al. 2014). Thus, these results imply that earthworms can affect the performance and the resource uptake of plants, with potential consequences for their competitive abilities and resistance to herbivory and disturbance, and so their persistence in the habitat, by altering different plant functional traits.

Grass aboveground productivity and reproduction benefits from interspecific competition and earthworms

Aboveground productivity of *P. palustris* monocultures and the mixed community was significantly increased in the presence of earthworms, while aboveground productivity of *S. laeve* and *V. americana* populations did not show any general short-term responses to the earthworm treatment. These results support the findings that the slow-growing species *S. laeve* and *V. americana* cannot exploit additional nutrients as rapidly as the fast-growing species *P. palustris* (Reich 2014), and provide only partial support for our hypothesis (1), expecting that plant productivity is generally increased by earthworm presence, even if belowground community productivity was overall higher for all species when earthworms were present. The grass community productivity may be more responsive to earthworm activity due to their high resource competitiveness and rapid growth (Eisenhauer and Scheu 2008b, Linder et al. 2018, Thouvenot et al. 2021), which is in line with the high aboveground biomass produced by the individuals of *P. palustris* irrespective of the treatment compared to the other species.

When growing in the interspecific community, several traits of *P. palustris* (lower C:N and LDMC but higher aboveground biomass, number of ramets and inflorescences) indicate an elevated nutrient uptake, growth and reproduction abilities comparing to monoculture, likely giving them a competitive advantage over other species. *Poa palustris* thus seems to

benefit from the presence of non-congeneric species in terms of biomass production, although other traits show opposite or no responses to treatments. Moreover, *P. palustris* developed more inflorescences when growing in earthworm presence. Inflorescences are an indicator for individual plant performance (Roscher et al. 2011), and an increased sexual reproduction may represent a competitive advantage (E-Vojtkó et al. 2020) rarely reported in previous studies on the consequences of earthworm invasion (Blume-Werry et al. 2020). As earthworms can change nutrient and water availability (Ferlian et al. 2020), they might act as a filter on plant reproduction (Eisenhauer et al. 2012, E-Vojtkó et al. 2020). Even though we found that *P. palustris* individuals benefit from earthworm presence and from interspecific competition (or missing intraspecific competition), we did not find any significant interaction effect between these two factors. Thus, we cannot conclude that earthworms induce a facilitative interaction between plant species.

Several functional traits of *V. americana* and *S. laeve* changed in interspecific competition (e.g. lower aboveground biomass, lower height growth, fewer ramets). For instance, the legume individuals developed a higher SLA in interspecific competition, which is a strategy to cope with lower light availability by raising their photosynthetic rate (Violle et al. 2007, Roscher et al. 2011). Nevertheless, we also did not find any interaction effect of plant diversity level and earthworm presence for any of the studied traits for these two species, providing little support for our hypothesis 2) expecting a negative effect of the high competition from grasses in the presence of earthworms on these two species. The finding that neither earthworm presence, nor a change of plant diversity level, had a significant effect on the C:N ratio of *V. americana* leaves supports results of former studies that state legumes are relatively independent of soil nutrient changes, e.g. caused by plant competition or the presence of earthworms (Wurst et al. 2003, Van Groenigen et al. 2014). Indeed, legume species are relatively independent of the nitrogen from the soil due to their symbiosis with *Rhizobium* species that fix N_2 in the root nodules of legumes (Hirsch et al. 2001, Eisenhauer and Scheu 2008b, Eisenhauer et al. 2009a), but can also be independent of the phosphorus (among other nutrients) when colonized by arbuscular mycorrhizal fungi (AMF) (Marschner and Dell 1994, Eisenhauer et al. 2009b, Hawkins et al. 2023). However, root nodulation was shown to be affected by soil fauna, including earthworms (Thompson et al. 1993, Doube et al. 1994), and invasive earthworm activities could disrupt the hyphal network (Jasper et al. 1989) and decrease the mycorrhizal colonization rate and the colonized root length (Lawrence et al. 2003, Paudel et al. 2016). This could affect nutrient uptake and performance of plants leading to a change in species dominance in the field. Thus, the low biomass produced by *S. laeve* and *V. americana* might have been a consequence of the effect of earthworms on one of these two root resource-acquisition strategies. The nodule production by the legumes, and the colonization by mycorrhizal fungi for all species that play critical roles in plant resource use and competition (Wen et al. 2022) in response to earthworm invasion, could thus be investigated in future studies.

Earthworm presence does not affect herbivory rate or herbivore survival

Neither herbivory rate, nor herbivore survival, of any of the plant species was affected by earthworm presence (hypothesis 4)). This lack of earthworm effects on herbivory rate and herbivore survival cannot be due to a too high initial soil nutrient status (Van Groenigen et al. 2014), as the initial C:N ratio of the soil used (20.98) was higher and the N content (0.06%) lower than in comparable studies (Wurst et al. 2005, Eisenhauer et al. 2007, Thouvenot et al. 2021). Contrary to the expectations that slow-growing species have a better defense than fast-growing species (Reich 2014), the grass species studied was not only the least palatable, but also the least suitable for the survival of herbivores, especially in a mixture. A possible explanation is that grass leaves contain silica, which enhances the abrasiveness of the tissues and reduces their digestibility (Vicari and Bazely 1993, Luyckx et al. 2017) by changing leaf surface morphology (Hall et al. 2020). These physical defenses would be enhanced in the presence of competitors. Furthermore, the increase of LDMC (i.e. higher defense for all plant species) and the decrease of C:N ratio (i.e. higher nutritious value of *P. palustris* and *S. laeve*) caused by earthworms could also have balanced each other out and, thus, prevented a change of herbivory rate. However, we note that herbivore effects might vary between the herbivore species according to their preferences, and further studies should thus investigate the effects of additional herbivore feeding guilds. Indeed, in addition to the herbivore feeding guilds (chewing, phloem-feeding, cell-feeding, etc.), multiple other aspects (e.g. density and diversity of earthworms, and plant functional group identity) were identified to have an impact on the outcome of earthworm effects on herbivory (Xiao et al. 2018).

Earthworm presence and competitive environment modulate herbivore effects on specific traits

We found differences in plant traits after a short-term aboveground herbivory (i.e. leaf area, SLA and RTD; hypothesis 3)). These effects were modulated by interspecific competition and earthworm presence, supporting our hypothesis 5) (i.e. effects of invasive earthworms and aboveground herbivory depend on plant community composition). Leaf area of plants growing in interspecific competition changed in the presence of herbivores, indicating that interspecific competition led to a leaf development being more sensitive to herbivory. Herbivores modulate the effect of earthworms on RTD of *P. palustris*. An increase in RTD is associated with higher resistance to pathogens and drought (Pérez-Harguindeguy et al. 2013), which might offer an advantage in tolerating herbivore attack by reducing the risk of other influences that may harm the herbivory-weakened plant (e.g. drought and pathogens). Further, a higher RTD might indicate translocation of resources after herbivore attack (Schwachtje et al. 2006, Babst et al. 2008, Gómez et al. 2010) or a more conservative use of, for example, carbon through lower root

exudation (Wen et al. 2022). We recommend future studies to investigate the relationship between plants, aboveground herbivory and detritivores by measuring belowground traits and physiological defense traits, using more plant species and functional groups to be able to derive a general framework regarding invasive earthworm impacts on biotic interactions and better predict the consequences for plant communities.

Implications for native understory plant communities and ecosystem functions

Our study shows that earthworms can affect plant functional traits and biotic interactions. These changes can have multiple consequences not only for plant community composition but also for ecosystem functioning. Although we cannot verify that the plant species studied here respond in the same way in invaded natural ecosystems in response to herbivores (note that only the effect of one generalist herbivore was tested here), and to the presence of earthworms, previous studies have measured some plant functional traits in the field. For example, invasive earthworms have been shown to induce changes in the height (Dávalos et al. 2015), nutrient content (Dobson et al. 2015) and the number of ramets (Dávalos et al. 2013) of some specific plant species. Further studies are needed to explore potential changes in species-specific traits and the herbivory rate of different plant and herbivore species. However, the observed changes in plant functional traits of the native plant community indicate that earthworms can affect plant development and resource acquisition in their natural habitat, and consequently biotic interactions. This could thus explain the changes in plant species dominance already observed in invaded plant communities (Nuzzo et al. 2009, Drouin et al. 2016, Alexander et al. 2022). Indeed, previous studies reported a decrease of conservative species belonging to the *Asteraceae* or *Violaceae* families (Alexander et al. 2022) or woody plants (Nuzzo et al. 2009), while Drouin et al. (2016) showed a grass dominance in the presence of a high density of invasive earthworms. According to the mass ratio hypothesis, the shift in plant species dominance and plant community trait composition are likely to impact ecosystem functioning, given that the most dominant species drives these functions. If earthworms foster the dominance of grass species in the plant community, we can expect the community traits to shift toward traits related to a more acquisitive strategy (i.e. high leaf nitrogen content, SLA and SRL, as well as a reduced height and low LDMC) in the presence of invasive earthworms. Consequently, the plant community may become more palatable and subject to herbivory (high N and low C). Furthermore, the litter produced (low LDMC, high SLA and N) would be more likely highly decomposable, and thus the decomposition process would be faster following earthworm invasion (Garnier et al. 2004, Pakeman et al. 2011, Pérez-Harguindeguy et al. 2013). However, on the other hand, grass-dominated plant communities could be less prone to herbivory due to high silica levels (Massey et al. 2006) and thus litter might be less palatable for the decomposer community (Vogel et al. 2013). To test these partly contradicting

hypotheses, studies are needed that investigate the changes in plant community trait composition under earthworm invasion and the resulting functional consequences.

Conclusions

Our study provides experimental evidence that invasive earthworms affect plant resource acquisition and competitive abilities by inducing changes in above- and belowground plant functional traits. These effects were partly modulated by competition and herbivory, and may reveal some of the mechanisms behind plant community changes in northern North American forests after earthworm invasion (Craven et al. 2017). Earthworms not only enhanced the aboveground biomass of the grass *P. palustris* but also improved its competitive advantage, such as reflected by a higher number of inflorescences, as well as elevated LDMC and tissue N content. Our findings thus confirm that the grass species *P. palustris* is a strong competitor and benefits from earthworm presence, at least in the short term. The herb and legume species did not benefit to a similar extent by earthworm presence, which is why we assume that earthworms increased the competitive strength of the grass species at the expense of the other plant functional groups. Earthworms did not affect herbivory rate and herbivore survival, but herbivory modulated the earthworm effect on RTD of *P. palustris* and on the SLA of *V. americana*. Our study shows that changed plant trait expression by earthworms is partly depending on the biotic context of the plants, i.e. the competitive environment and herbivory. Taken together, the results of the present study thus shed light on trait-based mechanisms potentially underlying observed shifts in plant community composition in northern North American forests, to the benefit of grasses and at the expense of legumes.

Author contributions

Rike Schwarz: Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal); Writing – original draft (lead); Writing – review and editing (lead). **Nico**

Eisenhauer: Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal). **Olga Ferlian**: Conceptualization (equal); Methodology (equal); Writing – review and editing (equal). **Fernando T. Maestre**: Resources (equal); Writing – review and editing (equal). **Benjamin Rosenbaum**: Methodology (equal); Writing – review and editing (equal). **Henriette Uthe**: Methodology (equal); Writing – review and editing (equal). **Lise Thouvenot**: Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Supervision (lead); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the iDiv Data Repository: https://doi.org/10.25829/idiv.3553-4jhr86 (Schwarz et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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