Plant-soil interactions of an invasive plant species and its non-invading congener differ in soil from their original range

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Abstract

The aim of this study was to compare plant-soil feedback (PSF) of globally invasive *Cirsium vulgare* in its native range with its non-invading congener *C. oleraceum*.

We assessed changes in soil nutrients and biota following soil conditioning by each species and compared performance of plants grown in self-conditioned and control soil, from which all, some or no biota was excluded.

The invasive species depleted more nutrients than the non-invasive species and coped better with altered nutrient levels. The invasive species had higher seedling emergence which benefited from the presence of unconditioned (non-specific) microbes. Biomass of the invasive species increased less in presence of self-conditioned microbiota and decreased more in presence of self-conditioned larger-sized biota compared to unconditioned biota than biomass of the non-invasive species. The invasive species showed greater ability to decrease its root-shoot ratio in presence of harmful biota and thus reduce their negative effects on its performance.

The results show that the invasive species is more limited by self-conditioned pathogens in the native range and benefits more from unconditioned mutualists, and thus may benefit more from loss of specialized soil biota in a secondary range. Our study highlights the utility of detailed PSF research in the native range of species for understanding the factors that regulate performance of invasive and non-invasive species in their native range, and for pinpointing the types of biota involved in their regulation and how this regulation changes across the plants life cycle.

Introduction

Understanding the success of invasive species and why some alien plants become invasive while others fail is a fundamental goal in the field of invasion ecology. Despite a high number of hypotheses explaining the success of invasive species, such as the enemy release hypothesis (Enders et al. 2020; Keane and Crawley 2002), we are far from fully comprehending what drives successful invasion. A promising approach to understanding the mechanisms that allow for invasion is to understand the factors that regulate species performance in the native range. For example, in their native range, invasive species might be those that take advantage of resource-rich environments by rapidly taking-up and depleting available resources, but then become limited by specialized enemies, e.g. various soil pathogens, once they become abundant. In their secondary range, these species might be less limited by specialized soil-borne enemies leading to possibly less negative conspecific plant-soil feedback, giving them advantage over other species (Aldorfova et al. 2020; Klironomos 2002; Kulmatiski et al. 2008; Suding et al. 2013).

Plant-soil feedback (PSF) is defined as abiotic and biotic changes of soil by plants that subsequently alter plant growth (Bever et al. 1997). PSF has been suggested to play a role in plant invasions. Invasive species often create more positive (or less negative) PSF compared to native species (Chiuffo et al. 2015;

Engelkes et al. 2008; Klironomos 2002; van der Putten et al. 2007) or non-invasive alien species (Aldorfova et al. 2020). Further, PSF has been shown to be more positive (or less negative) in the introduced compared to the native range of the plant species (Callaway et al. 2011; Reinhart and Callaway 2004; Reinhart et al. 2003). However, more studies in the native range are necessary to determine if plant performance of invasive plants is regulated by specialized enemies in their native range. Patterns by which plant species interact with the soil and soil biota in their native ranges might make some of them more likely to benefit from pathogen release when introduced to the secondary range than others. In support of this hypothesis, Zuppinger-Dingley et al. (2011) showed on a set of 16 grassland species that species invasive in some region of the world show more negative PSF in their native ranges than their non-invading relatives.

There are many different taxa involved in the biotic component of PSF, including bacteria, arbuscular mycorrhizal fungi (AMF), non-mycorrhizal fungi, protozoans, nematodes and microarthropods, and these groups can all have positive, negative or net neutral effects on the plants. Bacteria and non-mycorrhizal fungi are primarily associated with negative PSF effects however, they may enhance plant performance as well, either via direct growth promotion in the case of plant growth promoting rhizobacteria (Vacheron et al. 2013; van Loon 2007), or via increasing decomposition rates and nutrient availability (Weidner et al. 2015). On the contrary, AMF are usually considered to be beneficial for the plants, however, these may act as pathogens and reduce plant performance, especially in highly productive environments (Johnson et al. 1997). Soil mesofauna, comprising nematodes or various microarthropods, vary in their effects on plant performance, including negative effects of pathogens or root feeding organisms as well as positive effects of detritovores or organisms feeding on harmful microbiota (Bonkowski et al. 2009). Ideally, PSF experiments should identify the individual groups of soil biota that are involved in positive and negative interactions with plants (Dawson and Schrama 2016). In order to assess how specific soil biota affect plant performance, one would need to isolate the biota, prepare pure cultures and inoculate plants with them, which is extremely time consuming (Dawson and Schrama 2016). An easier, yet valuable approach is to take advantage of the fact that soil biota largely varies in size and inoculate the soil with whole or partial soil biota obtained by filtering soil solutions. By doing so, one can for example separate the effects of soil microbiota (fungi and bacteria) and soil mesofauna (van de Voorde et al. 2012; Wang et al. 2019a; Wang et al. 2019b).

Here, we quantified PSF-related mechanisms of invasion of *Cirsium vulgare* (Asteraceae), a species that is native to Europe, but has successfully naturalized on every continent except Antarctica, and is highly invasive in some areas (Julien and Griffiths 1998; Tenhumberg et al. 2008). We compared plant-soil interactions of *C. vulgare* (hereafter the invasive species) in its native range in Europe with plant-soil interactions of its native congener, *C. oleraceum*, that is not known to be naturalized or invasive anywhere in the world (hereafter referred to as the non-invasive species). Specifically, we used structural equation modeling to understand how the abiotic and biotic pathways in PSF influence plant performance. This requires quantifying how the species condition the soil, both in respect to changes in soil nutrient levels and composition of soil biota, how plant performance responds to soil conditioning, and which groups of soil biota (e.g., bacteria, fungi, AMF) are driving these changes in plant performance. Most studies

address PSF solely in terms of aboveground biomass of the plants as it is the easiest measure of plant performance (Kardol et al. 2013). However, it has been shown that PSF can change in intensity and even in direction throughout plant's life (Aldorfova et al. 2020; Dudenhoffer et al. 2018; Florianova and Munzbergova 2018) and that PSF depends on, and thus probably also affects, the size of the root system and allocation to root biomass (Aldorfova and Munzbergova 2019; Bergmann et al. 2016; Cortois et al. 2016). We therefore used three measures of plant performance: seedling emergence, aboveground biomass, and root-shoot ratio.

Material And Methods

Studied species and seed collection

For this study, we selected a pair of congeneric species, *Cirsium vulgare* (the invasive species) and *Cirsium oleraceum* (the non-invasive species), Asteraceae, Carduoideae. Both species are native to Europe, with one of their ecological optima being in ruderal vegetation. Specifically in the Czech Republic, *C. oleraceum* is more frequent and abundant than *C. vulgare* [occurrence frequency in vegetation plots 4.1% vs 1%, mean percentage cover 7.8% vs. 2.3%, and maximum percentage cover 88% vs. 38% (Wild et al. 2019)]. However, globally, and especially in North America, *C. vulgare* is reported to be a noxious weed and highly invasive species (Julien and Griffiths 1998; Sieg et al. 2003; Tenhumberg et al. 2008), while *C. oleraceum* has never been reported as an invader outside the native range.

Seeds of both species were collected in the field in the Czech Republic in 2017. For each species, we collected mature seeds from at least 10 individuals. Seeds from all individuals were mixed and mother plants were not further distinguished in the experiment. All collected seeds were surface sterilized with 10% dilution of SAVO Originál (a disinfectant based on 4.7% sodium hypochlorite) prior to the experiment to reduce the chance of soil contamination via seed surface fungi, as commonly done in experiments (e.g., Gallery et al. 2007; Oono et al. 2020).

Experimental design

Following a commonly used methodology (Bever et al. 1997; Kulmatiski et al. 2008), the plants were grown in a two-phase experiment. In the first (conditioning) phase, conditioned soil was prepared. Soil biotic and abiotic characteristics were assessed after the conditioning phase to compare the effect of the two species on the soil. In the second (feedback) phase, we studied conspecific PSF by growing the plants in 12 different types of soil, including conditioned and unplanted control soil with full, partial or no soil biota. This allowed us to quantify both abiotic and biotic PSF, as well as to distinguish between net effects of soil microbiota / total soil biota (presence of partial / full soil biota vs in soil with no biota) and specificity of soil biota (self-conditioned soil biota vs unconditioned soil biota from control soil), see more details below and in Table 1.

Conditioning phase

The aim of the conditioning phase was to prepare the soil, conditioned by the species, for the upcoming feedback phase. The conditioning phase was carried out between April 2018 and July 2018 in the experimental garden of the Institute of Botany of the Czech Academy of Sciences (49°59'38.972"N, 14°33'57.637"E), 320 m above sea level, temperate climate zone, where the mean annual temperature is 8.6 °C and the mean annual precipitation is 610 mm.

To set up the conditioning phase, we used a mixture of topsoil (purchased from JENA company) and sand (AGRO Jesenice) in 1:1 ratio. For chemical characteristics of the soil mixture see Table S1. For each species, we used 150 1-liter pots (10×10×10 cm) in the conditioning phase. Half of the pots were sown with 10 seeds of one of the species in April 2018, the other half of the pots remained unsown and served as controls. Even though the control pots remained unplanted during the conditioning phase, the soil was still a live soil in which a mixture of grassland plant species was previously grown, and it thus contained species non-specific soil biota. The soil originated in Central Bohemia, Czech Republic (i.e. the region of seed collection), but its exact history, such as which plant species were grown in it and for how long, has not been provided by the company. Each pot with conditioned soil was randomly assigned its unplanted control pot. The pairs of pots were always kept in close proximity to each other throughout the experiment so that they were exposed to exactly the same conditions. Both pots with and without plants were kept under the same conditions, regularly watered with tap water, and weeded on a weekly basis to avoid any effects of other species on the soil.

After the seeds germinated and the seedlings emerged, pots were thinned to one largest seedling. The soil was conditioned for 12 weeks, similar to a range of previous studies (e.g., Chiuffo et al. 2015; Florianova and Munzbergova 2018; Meijer et al. 2011; van de Voorde et al. 2011; van Grunsven et al. 2007; van Grunsven et al. 2010). Twelve weeks should be a sufficient time for the soil to get properly conditioned, particularly for small pots we used in our study, as all of the soil was thoroughly colonized by the roots by the end of the 12th week. A previous study showed that PSF is getting more negative with increasing the duration of the conditioning phase only up 6 weeks of conditioning (Lepinay et al. 2018). In addition, it has been shown that the initial changes in soil microbial composition very well reflect the long-term changes (Hannula et al. 2021). After the 12 weeks, in July 2018, all plants were harvested, divided into aboveground and belowground parts (all larger roots were carefully taken out from the soil by hand), dried to a constant weight, and weighed.

After the harvest, the pots with each species as well as their paired unplanted control pots were randomly divided into ten groups of 7-8 pots and their soil was mixed. For each species we thus had 10 sets of conditioned soil and 10 paired sets of control soil. Soil set served as a replicate and was further treated as such. From each soil set, one sixth of the soil was collected for analysis of soil chemistry and soil biota, one third was kept untreated to serve as source of specific biota for soil inoculation in the feedback phase, and the rest was sterilized by gamma irradiation (sterilization dose 25 kGy, performed by Bioster a.s. in Veverská Bítýška, Czech Republic) and used as a background soil in the feedback phase.

Feedback phase

The feedback phase was carried out between September 2018 and March 2019 in a greenhouse of the Institute of Botany of the Czech Academy of Sciences. The greenhouse was heated to 18°C and daylight was extended by two hours every day.

In the feedback phase, we grew each species in six treatments of conditioned soil and six treatments of unplanted control soil. These treatments included sterilized soil, sterilized soil inoculated with microbial filtrate of conditioned or control soil (i.e. self-conditioned vs unconditioned microbiota), sterilized soil inoculated with whole inoculum of conditioned or control soil, and non-sterilized whole soil (Fig. 1). Inoculum and filtrate always originated from the same soil set as the sterilized background soil or from the paired soil set with different soil conditioning. Using these treatments, we can assess the role of individual components in the PSF (Table 1).

To set up the feedback phase, we used 10 1-liter pots (10×10×10 cm) per species, soil conditioning and treatment, resulting in 120 pots per species and 240 pots in total. The bottoms of the pots were covered with expanded clay pebbles (keramzit) sterilized in autoclave up to the height of 2 cm to compensate for soil lost during the harvest of the conditioning phase, and the rest of the pots was filled with 500 ml of soil mixed depending on the treatment. For non-sterilized whole soil treatments, we used untreated soil from the conditioning phase of the experiment. For whole inoculum treatments, we mixed sterilized soil and untreated soil from the conditioning phase in a 9:1 ratio. For the treatments with microbial filtrate, we filled the pots with sterilized soil and we watered them with the microbial filtrate. The filtrate was created by mixing 50 ml of untreated soil in 500 ml of distilled water, homogenizing the mixture, and filtering it through two filter papers with pore size of 11 μm. Therefore, the microbial filtrate does not contain micro-arthropods, nematodes, or arbuscular mycorrhizal fungi, whereas it should contain soil bacteria and fungi (van de Voorde et al. 2012). For sterilized treatments, we filled the pots with sterilized soil and we watered them with autoclaved microbial filtrate.

Each pot was sown with 9 seeds of the same species as in the conditioning phase. The pots were kept in the greenhouse, regularly watered, and weeded when needed. All pots originating from one pair of soil sets were kept in the same block within the greenhouse. Number of emerged seedlings was recorded on a weekly basis. Three weeks after the first seedlings emerged in all pots, all seedlings but the largest one were removed from each pot to avoid competition. All seedlings emerging afterwards were recorded and removed as well. Twelve weeks after germination, the plants were harvested, divided into above- and below-ground biomass and weighed. All pots of both species were harvested at the same time.

Soil characteristics

Soil characteristics (i.e. soil abiotic characteristics, soil microbial community, and infection potential of AMF, see below) were analyzed after the conditioning phase for three types of soil: soil conditioned by the invasive species, soil conditioned by the non-invasive species, and the control soil. For each of the soil conditioning types, samples from six out of the ten soil sets were randomly selected for the analyses. In addition, the analyses were performed also on soil collected before the conditioning phase (Table S1).

For abiotic soil characteristics, we measured actual pH (pH measured in deionized H_2O) and exchangeable pH (cation exchange capacity), total C, N, P and available P, Ca, Mg, and K. For biotic characteristics, we determined soil microbial community composition using phospholipid and neutral fatty acids analysis (PLFA and NLFA) and we assessed the infection potential of AMF by commonly used procedures: the most probable number [MPN (Adelman and Morton 1986; Wilson and Trinick 1983)] and mean infection percentage [MIP (Giovannetti and Mosse 1980; Moorman and Reeves 1979)].

Abiotic soil characteristics

Actual and exchangeable pH was measured using deionized water and 0.1M solution of KCl as extracting solutions, respectively (ISO 10390: Soil quality – Determination of pH. International Organization for Standardization, ISO 2000). Total C and N contents were determined by methods of Ehrenberger and Gorbach (1973) using CHN catalyst (Carlo Erba NC 2500), total P was measured according to the method of Olsen and Sommers (1982). Available P was measured in filtrate of 5 g of air dried soil with 50 ml of 0.5M K_2SO_4 solution by flow injection analysis with spectrophotometric detection using the instrument QuikChem FIA + 8000 Series (Ammerman 2001; Egan 2001). Concentrations of available Ca^{2+} and K^+ were measured using atomic emission spectrometry method and available Mg^{2+} using atomic absorption spectrometry according to methods of Moore and Chapman (1986) and (Dědina 1987), with solution of 1M ammonium acetate as the extractant. All analyses were performed by the Analytical Laboratory of Institute of Botany of the Czech Academy of Sciences in Průhonice.

Soil microbial community

Soil microbial community composition was assessed using PLFA analysis performed by the Laboratory of Environmental Biotechnology, Institute of Microbiology of the Czech Academy of Sciences, following the methodology described in Garcia-Sanchez et al. (2019). The PLFA were extracted from 1 g of freeze-dried soil samples with a mixture of chloroform-methanol-phosphate buffer (1:2:0.8, v/v/v), as previously described by Bligh and Dyer (1959). The lipids were fractionated into neutral lipids (NLFA), glycolipids and polar lipids (PLFAs) using an extraction cartridge (LiChrolut Si-60, Merck, White-house Station, USA), and NLFA and PLFA were subjected to mild alkaline methanolysis as described in Snajdr et al. (2008). The free methyl esters of NLFA and PLFAs were analyzed by gas chromatography-mass spectrometry (450-GC, 240-MS ion trap detector, Varian, Walnut Creek, CA) following the same procedure described by Sampedro et al. (2009).

The soil microbial community composition was characterized using the following PLFAs: fungal biomass was estimated on the basis of 18:2w6,9 content (Snajdr et al. 2008), bacterial biomass was quantified as the sum of i14:0, i15:0, a15:0, 16:1w5, 16:1w7; 16:1w9, 10Me-16:0, i16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 18:1w7, 10Me-18:0, and cy19:0. Actinobacterial biomass was determined as the sum of 10Me-16:0, 10Me-17:0, and 10-Me18:0, Gram-positive bacteria (G+) as sum of i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0, and Gram-negative bacteria (G-) as the sum of 16:1w7, 16:1w9, 18:1w7, cy17:0, and cy19:0. The NLFA 16:1w5 was assigned as a marker for the quantification of AMF and total PLFA concentration was

used to estimate the total viable microbial biomass (Olsson et al. 2003). Last, we calculated microbial ratios F:B (fungi : bacteria), G+:G- (Gram-positive bacteria : Gram-negative bacteria), and F:AMF (fungi : AMF).

Infection potential of AMF

Infection potential of AMF was assessed by MIP and MPN methods. In the MIP assay, the colonization intensity of AMF is measured after a certain period of bait plant cultivation and the index of root colonization is the percentage of the number of 1-cm root segments showing detectable AMF colonization (Moorman and Reeves 1979). In MPN method, the test plants are grown in serial dilutions of the inoculum and the propagule density of the original material is statistically calculated from MPN scores (Feldmann and Idczak 1992).

To assess MPN and MIP, we evaluated mycorrhizal colonization of maize roots [standardly used for assessing MPN and MIP as its roots are strongly colonized by AMF (Moorman and Reeves 1979)] that was grown in each of the studied types of soil in 1:0, 1:10, 1:100, 1:1000, 1:10000 dilutions with soil sterilized in autoclave, in five replicates per dilution. Maize seeds (*Zea mays* convar. *saccharata*, var. Ashworth) were purchased from a commercial supplier (ReinSaat KG company, St. Leonhard am Hornerwald, Austria), they were germinated in Petri dishes in sterile conditions, replanted into 100 ml plastic containers (4×14 cm), and left growing in a greenhouse. After six weeks, the plants were harvested, fine roots from the middle part of the root system were collected, placed in 10% KOH for three months to bleach, and stained (left for 12h in 2% lactic acid, 12h in 0.05% trypan blue in lactoglycerol, rinsed in water, and soaked into lactoglycerol prepared from glycerol, 80% lactic acid and distilled water in 3:2:5 ratio).

The stained roots from 1:10, 1:100, 1:1000, and 1:10000 dilutions were observed using a binocular magnifier and presence of AMF propagules was recorded. MPN/ml was calculated using a program MPN Calculator, Build 23 using information on types of dilutions, number of replicates per dilution and number of replicates per dilution in which AMF propagules were recorded. To assess MIP, only the 1:10 dilution was used. Stained roots were placed into a Petri dish with a 1x1 cm grid and presence of AMF propagules at 200 intersections of roots with the grid was recorded using a binocular magnifier. An average value from the five replicates was calculated both for MPN and MIP, resulting in one MPN and one MIP value per soil sample and six replicates per soil conditioning type.

Statistical analyses

Differences in soil biotic and abiotic characteristics between soil conditioned by *C. vulgare*, and by *C. oleraceum* were studied using linear direct gradient analysis (Redundancy Analysis, RDA) and Monte-Carlo permutation tests (Ter Braak and Šmilauer 2012) with 499 permutations. Dependent variables used in this analysis were all the studied soil characteristics except for actual pH, K content, total microbial and bacterial biomass, which were excluded due to high correlations with other variables (Table S2). The variables were standardized prior to the analysis. The independent variable was conditioning species. We

repeated the analysis with all three soil conditioning types including the control soil and we present the results in the appendix (Fig. S1). The analyses were performed in Canoco 5 (Ter Braak and Šmilauer 2012). As a supplementary analysis, we also performed ANOVA using R 3.6.1 (R Core Team 2019) always with one of the studied soil characteristics as dependent variable and we tested the differences between pairs of group means using Tukey post hoc tests (Fig. S2).

Differences in plant performance between individual treatments and soil conditioning types in the feedback phase were tested using a linear (square root transformed biomass and root-shoot ratio) or generalized linear (seedling emergence as number of emerged seedlings out of the number of seeds sown, with binomial error distribution) mixed effect models in the R package 'lmerTest' (Kuznetsova et al. 2017) with identity of the soil set as random effect, and species, soil conditioning, treatment (sterilized soil, filtrate from control soil, filtrate from conditioned soil, inoculum from control soil, inoculum from conditioned soil, non-sterilized whole soil), and their interactions as explanatory variables. To estimate p-values, we used F-tests comparing two models with and without a tested term, using a 'drop1' function in the 'lmerTest' package (Kuznetsova et al. 2017). To assess differences between pairs of group means, we used Tukey post-hoc tests adapted to mixed effect models using 'glht' function in 'multcomp' R-package (Hothorn et al. 2008).

Afterwards, we repeated the analysis using the type of soil biota (filtrate vs inoculum) and conditioning of soil biota (from control vs from conditioned soil) as explanatory variables instead of the treatment variable. We performed this analysis on a subset of data excluding the sterilized and the non-sterilized whole soil treatment, since in these treatments it is not possible to distinguish the effects of type and conditioning of soil biota. We obtained very similar results when including only the subset of treatments in the analyses and so we only present these results in the Results section. Results of the analyses including all the treatments and not differentiating between type and conditioning of soil biota can be found in the appendix (Table S3). The two treatments which are excluded from the main analyses are, however, visualized in some of the graphs and compared using multiple comparisons with the rest.

Last, we used structural equation modeling [performed in the 'lavaan' package (Rosseel 2012) in R] to assess how individual components of soil, i.e. amount of soil nutrients, bacterial, fungal and AMF biomass, affect biomass of the two species. For the analysis, we only used data on plant biomass from the non-sterilized whole soil treatment as detailed soil analyses are only available for this treatment. A separate model was created for each species. The assumed relationships were as follows: (i) plant performance is affected by the amount of soil nutrients and by bacterial, fungal and AMF biomass, (ii) bacterial, fungal and AMF biomass are affected by the amount of soil nutrients, and (iii) bacterial, fungal and AMF biomass are correlated.

Results

Effect of conditioning species on soil characteristics

Soil characteristics after the conditioning phase significantly differed between soils conditioned by the invasive and by the non-invasive species (Pseudo-F = 6.0, p = 0.004, 37.59% of explained variation, Fig. 2). Values of MPN and AMF biomass were higher in soils conditioned by the invasive species (Fig. 2, Fig. S2), and in both cases the values were much higher than in the control soil (Fig. S1, S2). Nutrient levels and both bacterial and fungal biomass were higher in soils conditioned by the non-invasive species (Fig. 2, Fig. S2).

Effect of soil conditioning and treatments on plant performance in the feedback phase

In the feedback phase, the invasive species had nearly four times higher overall seedling emergence and two times lower root-shoot ratio than the non-invasive species (Table 2, Fig. S3). The two species did not differ in overall biomass production (across all treatments, Table 2).

Soil conditioning (i.e. conditioning of the sterilized background soil) negatively affected plant biomass, with no differences between the two species (Table 2, Fig. S4). Type of soil biota (i.e. microbial filtrate, whole-soil inoculum, or whole soil) had a significant effect on plant biomass and root-shoot ratio (Table 2). In both cases, the values were the highest in microbial filtrate treatments, lower in whole soil inoculum and the lowest in whole soil (Fig. S5). The values were decreasing roughly by 25 % per treatment for biomass and by 15 % for root-shoot ratio. The effects did not differ between the two species (Table 2).

Effect of conditioning of soil biota, i.e. whether the biota originated from control or conditioned soil (filtrate or inoculum from control soil, filtrate or inoculum conditioned soil), on biomass and root-shoot ratio differed between the two species (Table 2). When considering both filtrate and inoculum together, the non-invasive species had nearly by 30% higher biomass when grown with conditioned biota compared to biota from control soil, while the invasive species performed similarly with both conditioned and control biota (Fig. 3a). The root-shoot ratio of the non-invasive species did not differ when grown with conditioned and control biota, while the invasive species slightly, but significantly decreased its root-shoot ratio when grown with conditioned biota.

The interaction between species, type of soil biota and soil biota conditioning was not significant for any of the measures of plant performance (Table 2). The interaction of species and treatment (comprising type and conditioning of biota) was significant for seedling emergence in the analysis using all treatments (Table S3). For the invasive species, seedling emergence in presence of microbial filtrate from control soil was approximately by 10 % higher (corresponding roughly to one extra seedling emerged) than in microbial filtrate from conditioned soils as well as the sterilized soil, but no differences in seedling emergence among the treatments were found for the non-invasive species (Fig. S6).

The interaction between species, soil conditioning, type of soil biota, and conditioning of soil biota was significant for biomass and root-shoot ratio (Table 2). In presence of conditioned microbial filtrate, the non-invasive species had higher biomass than in the control microbial filtrate in conditioned but not in control soil. The invasive species had higher biomass with conditioned filtrate than with control filtrate in control but not in conditioned soil (Fig. 4a). Root-shoot ratio was lower in plants grown with conditioned

soil inoculum than with control soil inoculum in both control and conditioned soil for the invasive species, but only in control soil for the non-invasive species (Fig. 4b).

Determinants of plant performance

Structural equation models (Fig. 5) showed that the determinants of plant performance in the non-sterilized whole soil treatment differ between the non-invasive and the invasive species. The non-invasive species responded negatively to bacterial biomass and positively to fungal biomass and soil nutrients levels, while the invasive species responded positively to bacterial biomass and was not significantly affected by fungal biomass or soil nutrients. While both species responded negatively to AMF biomass, only the invasive species significantly increased the AMF biomass as it depleted soil nutrients (Fig. 5).

Discussion

In the present study, we compared plant-soil interactions of two congeneric plant species native to Europe that differ in their invasive status outside their native range, the non-invasive C. oleraceum and the invasive C. vulgare. We showed that compared to its non-invasive congener, the invasive *C. vulgare* more rapidly depleted nutrients from the soil, was less influenced directly by the availability of soil nutrients, and responded less positively / more negatively in terms of biomass and root-shoot ratio to presence of self-conditioned soil biota compared to unconditioned biota from control soil. The invasive species also had significantly higher seedling emergence, which increases its chances for successful invasion regardless of plant-soil interactions. Our results suggest that plant-soil interactions may play a role in the invasive potential of *C. vulgare* and highlight that experimental studies from the native range on PSF can improve understanding of processes that regulate invasive plant populations.

Soils conditioned by the invasive species had lower levels of nutrients, in particular of available P, than soils conditioned by the non-invasive species (Fig. 2). This is in line with previous research showing that invasive species often exploit soil nutrients more efficiently than non-invasive species (Dassonville et al. 2008; Funk and Vitousek 2007; Sardans et al. 2017), allowing them to gain competitive advantage over other species. Importantly, despite lower nutrient levels in soils conditioned by the invasive species, this species did not show more negative abiotic PSF than the non-invasive species (Fig. 4a, comparison of performance in sterilized conditioned soil and sterilized control soil, i.e. grey columns, non-significant differences in both species). In line with that, the structural equation model showed lower sensitivity of the invasive species to soil nutrients than the non-invasive species (Fig. 5). This means that the invasive species copes better with altered nutrient levels, indicating its higher plasticity in response to nutrients, a common feature of successful invaders (Burns 2004; Daehler 2003; Funk 2008).

Both plant species had lower biomass when more types of soil biota were included (Fig. S5), showing that the negative effects of soil biota, in our study particularly the larger-sized soil biota, prevail over the positive effects, as has been shown in previous research (van de Voorde et al. 2012; Wang et al. 2019b). The invasive species had more negative biotic PSF driven by the larger-sized biota than the non-invasive species (Fig. 3, Fig. 4, comparison of performance in soil with inoculum conditioned vs inoculum control,

i.e. light blue vs dark blue columns, significant difference for invasive species in control soil, non-significant for non-invasive species) when defining PSF as a difference in performance of plants grown with biota from self-conditioned ('home') and control ('away') soil, as recommended when the research question concerns the specificity of soil feedback effects (Brinkman et al. 2010; Cortois et al. 2016). The results are in line with the finding of Zuppinger-Dingley et al. (2011) that potentially invasive species in their native range are held in check by more negative PSF compared to native species that do not become invasive elsewhere. It is unclear in our study if this result is due to the differences in composition of the self-conditioned biota (i.e. more specific enemies) or just their abundance since both are likely to differ from an unplanted control. Another limitation of our approach is that we did not inoculate the soil with soil collected at natural localities of the study species as usually done in PSF studies. Our soil therefore included non-specific soil biota in the beginning of the experiment which was transitioned into more species-specific communities during the conditioning phase, but these were still only a subset of what was originally found in the topsoil and sand mix used in the study and therefore did not need to reflect soil microbial community of either species in natural conditions. On the other hand, the situation simulates the introduction of the two species into a new environment, similarly to what they would experience within the process of invasion, and it therefore corresponds with the main focus of this study.

Bacterial biomass had an overall negative effect on the biomass of the non-invasive species, but positive on the invasive species (Fig. 5), suggesting that the invasive species was less affected by bacterial pathogens and benefited more from bacterial mutualists. There is a large chance that the species benefits from presence of bacterial mutualists in the secondary range as well since most mutualists are quite generalistic (Bronstein 2003) and invasive plants can often form mutualisms as effective or even more effective in the new ranges than in the old range (Parker and Gilbert 2007; Richardson et al. 2000). Interestingly, both species benefited from growth with their self-conditioned microbiota compared to the unconditioned control microbiota and this was more pronounced for the non-invasive species (Fig. 4a). This result may partially explain the differences in success of the two species when introduced into a secondary range. When moving to the secondary range, the plants are supposed to leave their specialized soil biota behind and only be affected by the local generalist (i.e. non-specialized) biota, which in case of the invasive *C. vulgare* seem to have more positive effects on its performance.

In contrast, the invasive species responded much more negatively to self-conditioned larger-sized soil biota compared to the control biota (Fig. 4, comparison of inoculum conditioned vs inoculum control), providing more opportunities for the invasive species to benefit from enemy release when introduced to the secondary range than for the non-invasive species. Larger-sized biota in our study refers to mesofauna such as nematodes and microarthropods, but also AMF. Since we only quantified AMF but did not study composition of the mesofauna, we cannot say which groups contributed the most to the negative PSF and the differences between species. AMF had a net negative effect on both plant species (Fig. 5). In the literature, more studies find net positive effects of AMF on plants, however, there are several other studies that report negative effects of AMF (Janos 2007; Johnson et al. 1997), particularly in nutrient-rich soils such as the soil used in our experiment. For the invasive species, biomass of AMF with negative effect on plant performance was negatively correlated with level of soil nutrients (Fig. 5).

Since the invasive species depleted nutrients from soil more efficiently than the non-invasive species (Fig. 2), it also accumulated more of the parasitic AMF. Even though AMF have relatively low host specificity, host preference in natural ecosystems has been identified (Sanders 2003). Because AMF composition differs between world regions (Sturmer et al. 2018), chances are that the invasive species leaves behind some of the parasitic AMF when moving to the secondary range, which would further contribute to its invasion success. However, since we do not have data on AMF species composition or on the effect of AMF on the species in its secondary range, this explanation remains purely hypothetical.

Root-shoot ratio of both species decreased when more types of soil biota were included (Fig. S5). In addition, the invasive species decreased its root-shoot ratio in presence of self-conditioned biota compared to unconditioned biota in case of the larger-size biota in whole-soil inoculum (Fig. 5b), pointing to its greater sensitivity to specialized biota and possibly greater plasticity in biomass allocation compared to the non-invasive species. Since the size of root system determines the intensity of interactions between plants and soil biota (Aldorfova and Munzbergova 2019; Bergmann et al. 2016; Cortois et al. 2016), reducing allocation into root biomass in presence of detrimental soil biota may serve as a protective mechanism for the plants, minimizing the negative effects of soil biota on plant growth. On the other hand, reduced allocation to roots may have negative effect on plant growth in the long term via reduced ability to absorb nutrients, in case nutrients are limiting, and more research is thus needed to understand the relationship between allocation to roots and plant-soil feedback.

Seedling emergence was overall higher for the invasive species and did not differ between whole soil inoculum treatment and sterilized soil for either species. Microbial filtrate from control, but not from self-conditioned soil, however, increased seedling emergence of the invasive species (Fig. S6). This suggest that non-specialized microbes, i.e. those the species is relatively more likely to encounter in the secondary range, benefit plant performance of the invasive species in early stages of their life, but that their positive effect is counterbalanced by specialized microbial pathogens when grown with self-conditioned microbiota from the native range. This, combined with the high overall seedling emergence, may be another factor contributing to the invasiveness of *C. vulgare*. However, our seedling emergence data were based only on 10 seeds sown per pot, so the results are not robust and should be interpreted with this limitation in mind.

Conclusions

By comparing plant-soil interactions of globally invasive *Cirsium vulgare* and its non-invading congener *C. oleraceum*, we showed that plant-soil interactions in their native range may help to explain the differences in the invasive success of the species. This invasive species is able to reduce nutrients to lower levels but maintain its high performance regardless of soil nutrient levels. While soil bacteria in general have more positive effect on the invasive species, the invasive species benefits less from growth with self-conditioned microbiota compared to unconditioned (control) microbiota. On the other hand, it is relatively more harmed by self-conditioned larger-sized soil biota. Since the self-conditioned biota is more likely specialized and therefore less likely to be present in the secondary range than the unconditioned

biota, our results suggest that the invasive species may benefit more from pathogen release and at the same time suffer less from loss of specialized mutualists when transferred to the secondary range than the non-invasive species. Further studies considering more complex interactions – such as experiments including interspecific PSF (Bever et al. 1997) along with conspecific PSF studied here or experiments combining PSF treatments with intra- and/or inter-specific competition treatments (Lekberg et al. 2018; Shannon et al. 2012) – are needed to account for context dependency of PSF studies and to provide a deeper insight into environmental constraints limiting performance of both species in their native and secondary ranges.

Declarations

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Tables

Table 1

Components of PSF observed by comparing individual treatments.

PSF component	Compared treatments	
total net PSF	conditioned non-sterilized whole soil	control non-sterilized whole soil
abiotic PSF	conditioned sterilized soil	control sterilized soil
total biotic PSF	soil with whole-soil inoculum	sterilized soil
microbial PSF	soil with microbial filtrate	sterilized soil
mesobiotic PSF	soil with whole-soil inoculum	soil with microbial filtrate
specificity of soil microbiota	microbial filtrate from conditioned soil	microbial filtrate from control soil
specificity of soil biota	inoculum from conditioned soil	inoculum from control soil

Table 2

Results of generalized linear (seedling emergence, binomial error distribution) or linear mixed effect models testing the effect of species identity, soil conditioning, type of soil biota (microbial filtrate, whole soil inoculum), conditioning of soil biota (from control or conditioned soil), and their interactions on seedling emergence, plant biomass and root-shoot ratio. Significant results ($p \leq 0.05$) are in bold, marginally significant ($p \leq 0.1$) in italic. Df represent Satterthwaite approximation for degrees of freedom. The analyses are based on a subset of data excluding the sterilized and the non-sterilized whole soil treatments. Results of the analyses of a full dataset are shown in Table S3

		seedling emergence		biomass		root-shoot ratio	
	df	F	p	F	p	F	p
species	1, 11	**52.21**	**<0.001**	0.65	0.433	**29.70**	**<0.001**
soil conditioning [soil]	1, 89	0.04	0.843	**21.10**	**<0.001**	**4.07**	**0.047**
type of soil biota [biota type]	1, 88	0.47	0.492	**30.99**	**<0.001**	**18.19**	**<0.001**
conditioning of soil biota [biota cond.]	1, 82	*3.85*	*0.050*	**4.13**	**0.045**	0.60	0.439
species x soil	1, 89	*3.72*	*0.054*	0.29	0.594	**7.03**	**0.009**
species x biota type	1, 88	0.09	0.767	0.53	0.469	0.16	0.692
soil x biota type	1, 85	**9.05**	**0.003**	0.18	0.672	1.27	0.263
species x biota cond.	1, 82	1.19	0.276	**5.60**	**0.020**	**4.23**	**0.043**
soil x biota cond.	1, 89	0.01	0.936	**4.81**	**0.031**	1.28	0.261
biota type x biota cond.	1, 83	2.33	0.127	**11.44**	**0.001**	**16.28**	**<0.001**
species x soil x biota type	1, 85	*3.68*	*0.055*	0.94	0.334	1.77	0.187
species x soil x biota cond.	1, 88	2.00	0.157	2.50	0.117	0.15	0.700
species x biota type x biota cond.	1, 83	2.68	0.102	0.19	0.665	2.35	0.129
soil x biota type x biota cond.	1, 84	0.08	0.778	0.04	0.841	1.76	0.188
species x soil x biota type x biota cond.	1, 84	1.44	0.230	**4.15**	**0.044**	**4.65**	**0.034**

Figures

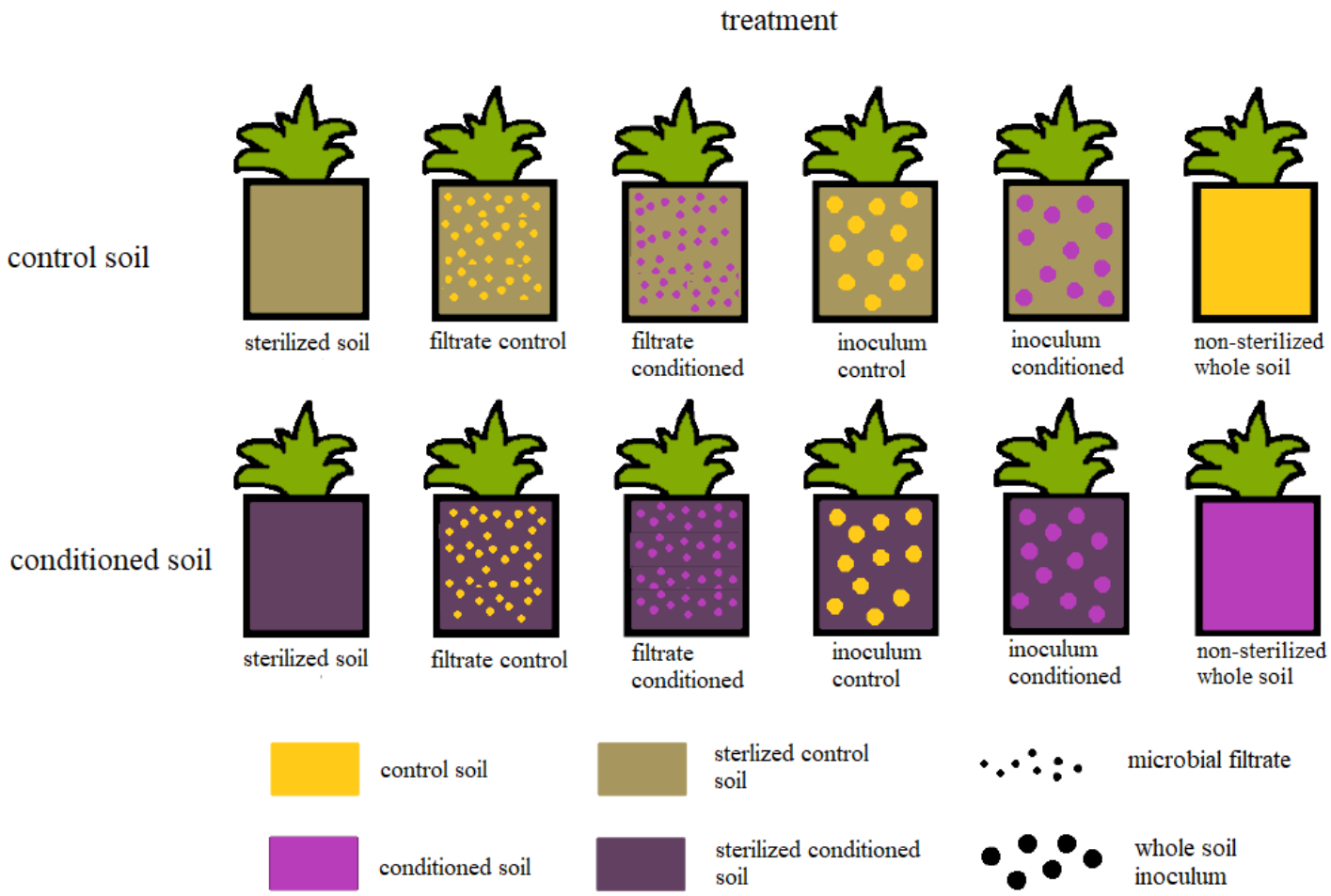

Figure 1

Schematic diagram of soil conditioning and treatments used in the feedback phase

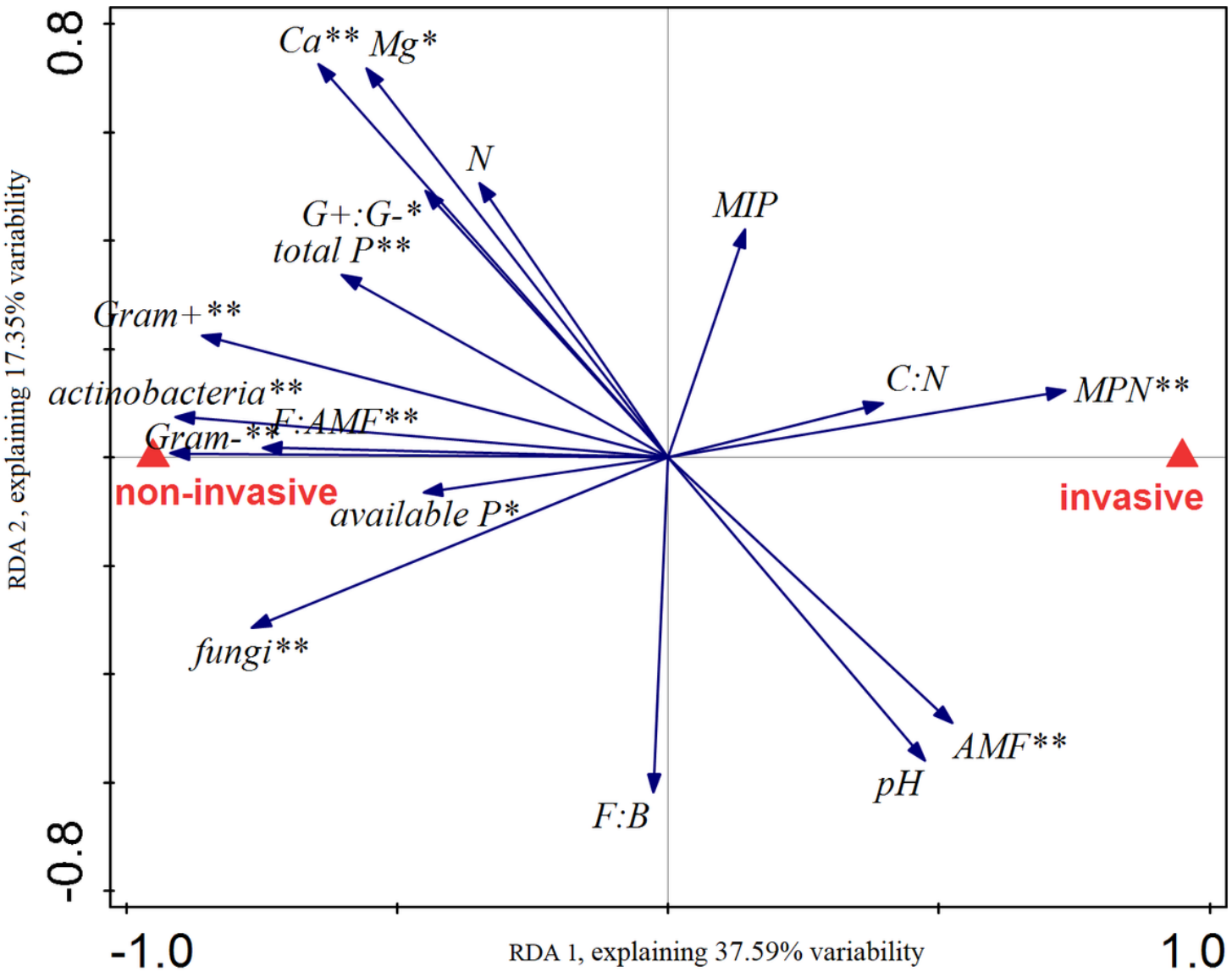

Figure 2

Differences in soil biotic and abiotic characteristics in soils conditioned by the invasive and the non-invasive species. Results displayed are an ordination plot based on RDA tested using a Monte-Carlo test with 499 permutations. Pseudo-F = 6.0, p = 0.004, the first two axes explained 37.59% and 17.35% variability in the data, respectively. Data are centered and standardized across soil characteristics. ** indicates characteristics that are significantly ($p < 0.05$) different between the two soil types in individual tests, * indicates marginally significant ($p < 0.1$) differences. Actinobacteria, Gram+, Gram-, fungi and AMF represent biomass of the groups obtained by PLFA/NLFA analyses. G+:G-, F:AMF, and F:B represent ratios of Gram positive (G+) and Gram negative (G-) bacteria, fungi (F), AMF and bacteria (B)

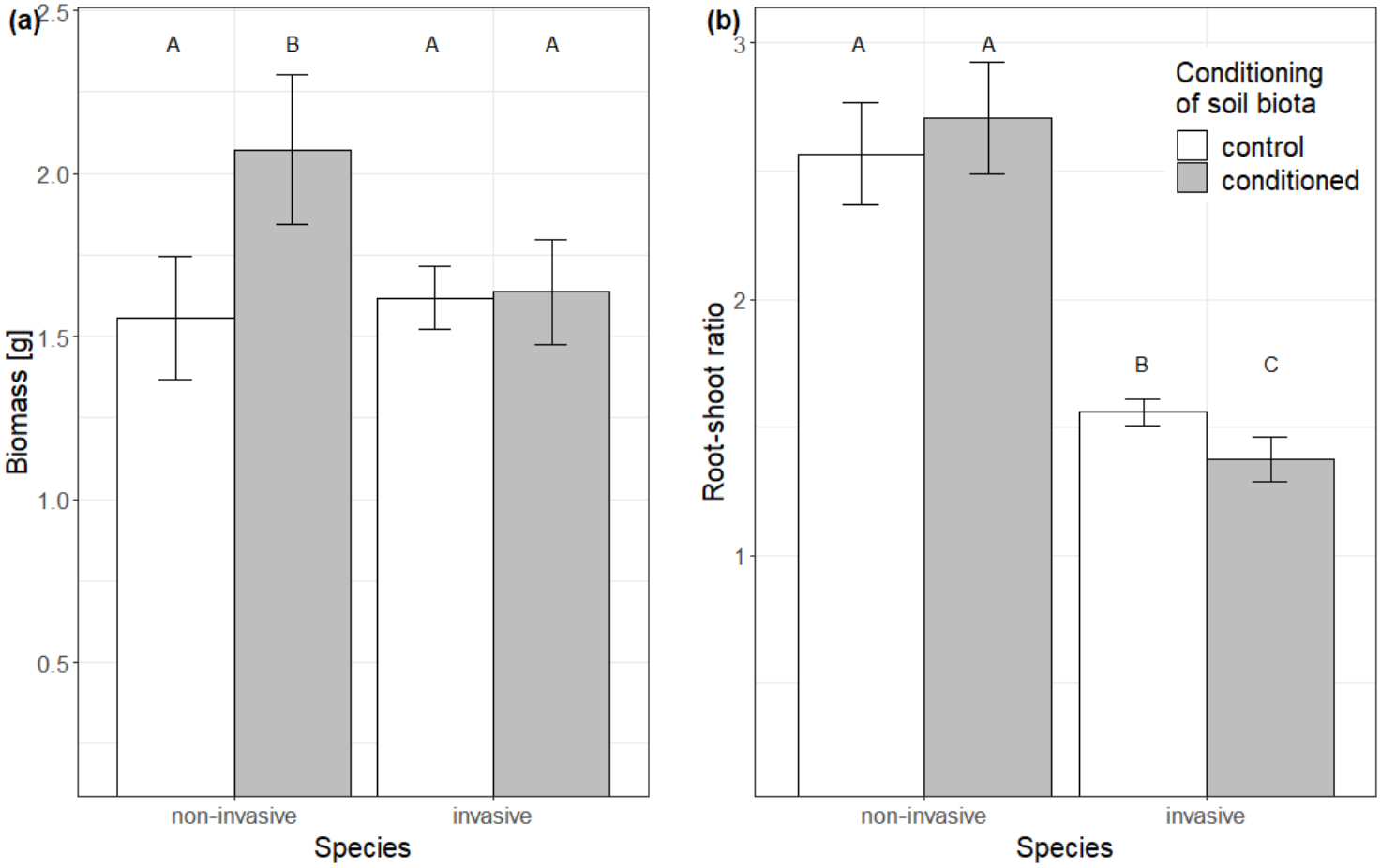

Figure 3

Effect of conditioning of soil biota on (a) biomass and (b) root-shoot ratio of the two study species. Bars and error lines represent mean ± SE. Bars that share the same letter do not significantly ($p > 0.05$) differ from each other after Tukey post-hoc tests

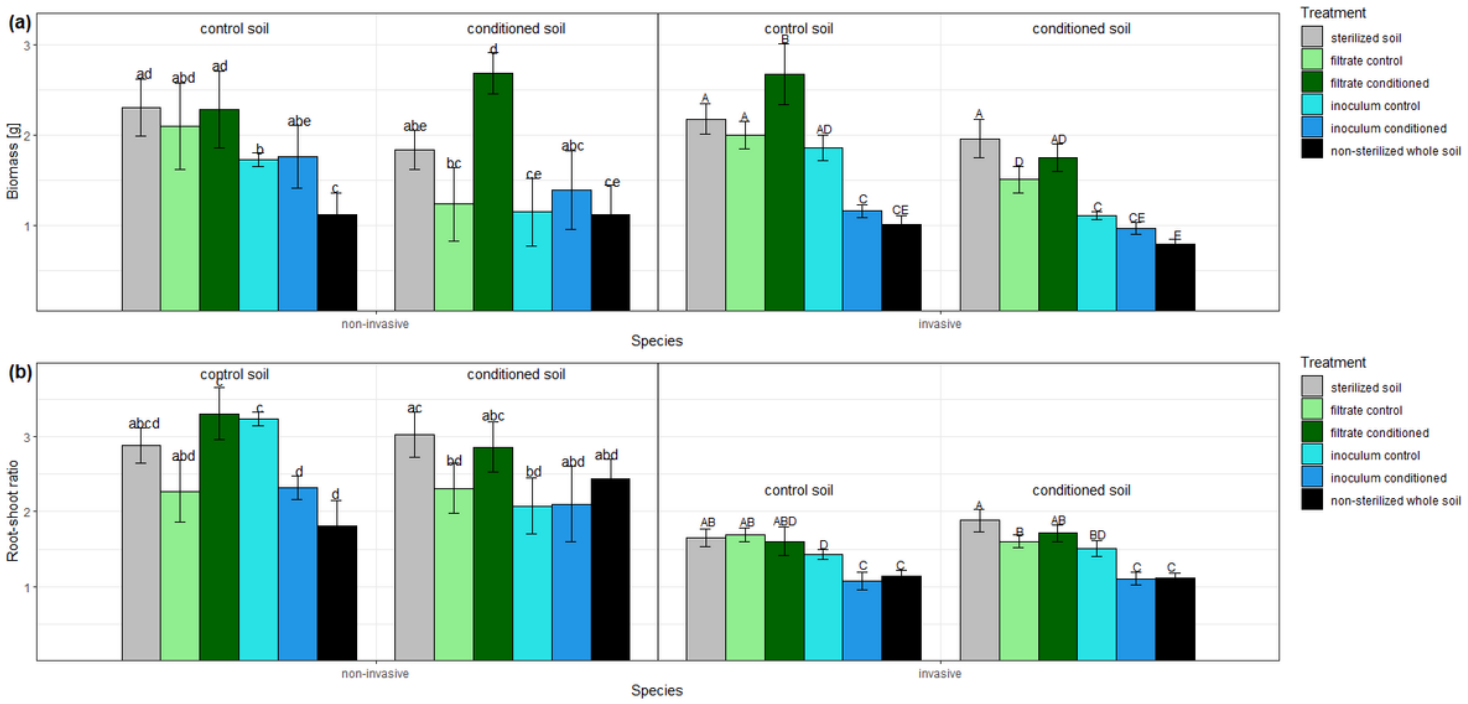

Figure 4

Effect of soil conditioning and treatment (type and origin of soil biota) on (a) biomass and (b) root-shoot ratio for individual species. Bars and error lines represent mean ± SE. Bars of one species that share the same letter do not significantly ($p > 0.05$) differ from each other after Tukey post-hoc tests

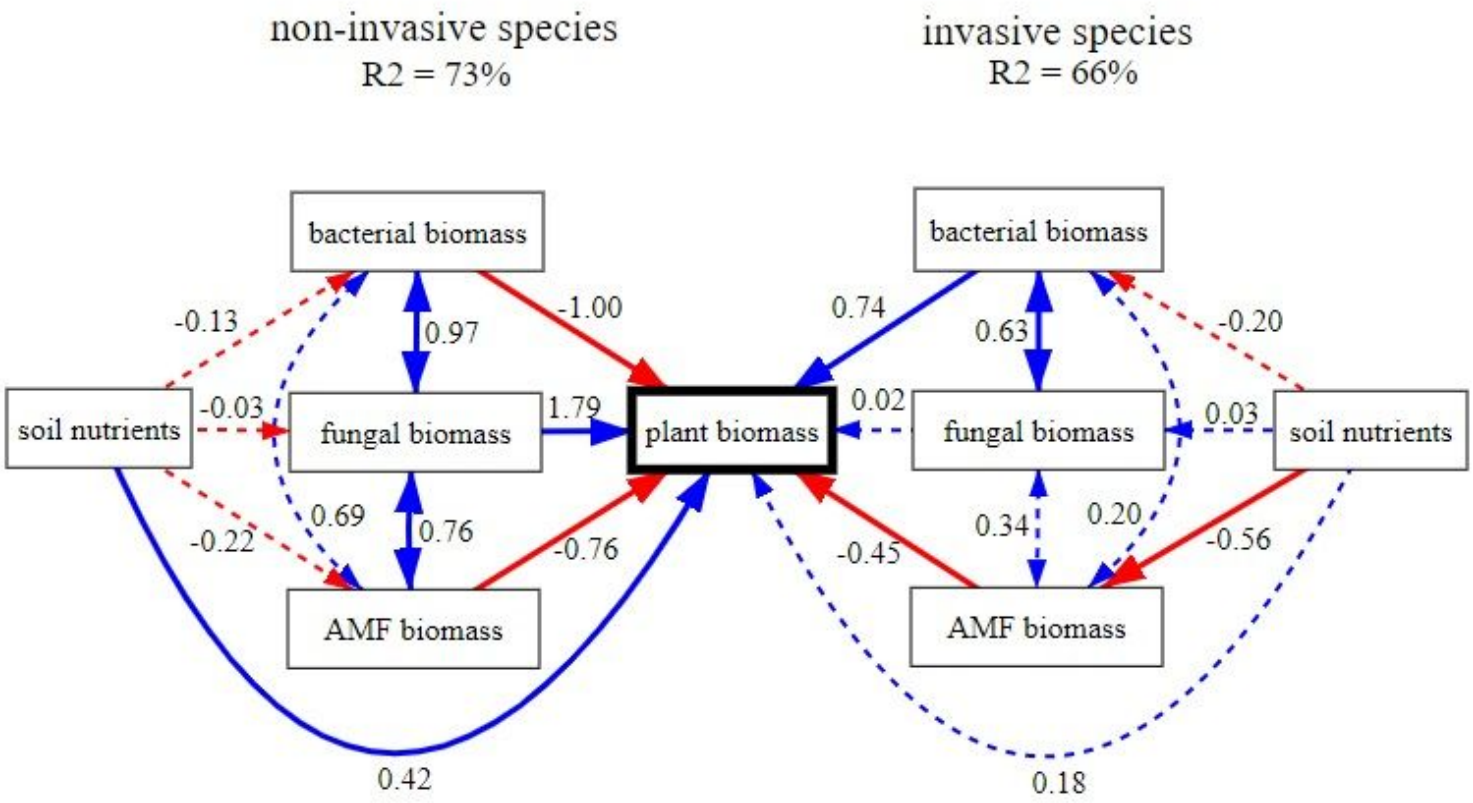

Figure 5

Structural equation model (path analysis) of soil characteristics influencing biomass of the non-invasive (panels on the left-hand side) and the invasive species (panels on the right-hand side). Red arrows indicate negative relationships, blue arrows positive relationships. Solid arrows indicate significant relationships ($P < 0.05$), dashed arrows non-significant relationships ($P > 0.05$). Standardized path coefficients are shown

Supplementary Files

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