Received: 15 June 2020 | Accepted: 15 December 2020

DOI: 10.1111/1365-2745.13584

RESEARCH ARTICLE

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

Fungal root endophytes influence plants in a species-specific manner that depends on plant's growth stage

Stefan Geisen[1,2] | Freddy C. ten Hooven[1] | Olga Kostenko[1] | L. Basten Snoek[1,2,3] | Wim H. van der Putten[1,2]

[1]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

[2]Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands

[3]Theoretical Biology and Bioinformatics, Utrecht University, Utrecht, The Netherlands

Correspondence
Stefan Geisen
Email: stefan.geisen@wur.nl

Funding information
ERC-ADV 323020; Netherlands Organization for Scientific Research; NWO-VENI, Grant/Award Number: 016.Veni.181.078

Handling Editor: Franciska de Vries

Abstract

1. The mycobiome (fungal microbiome) influences plants—from seed germination to full maturation. While many studies on fungal-plant interaction studies have focused on known mutualistic and pathogenic fungi, the functional role of ubiquitous endophytic fungi remains little explored.
2. We examined how root-inhabiting fungi (endophytes) influence range-expanding plant species. We isolated endophytes from three European intra-continental range-expanders and three congenerics that are native both in the range expander's original (southern Europe) and new (northern Europe) range. To standardize our collection, endophytes were obtained from all six plant species growing under controlled conditions in northern (new range of the range expander) and southern (native range of the range expander) soils. We cultivated, molecularly identified and tested the effects of all isolates on seed germination, and growth of seedlings and older plants.
3. Most of the 34 isolates could not be functionally characterized based on their taxonomic identity and literature information on functions. Endophytes affected plant growth in a plant species–endophyte-specific manner, but overall differed between range-expanders and natives. While endophytes reduced germination and growth of range-expanders compared to natives, they reduced seedling growth of natives more than of range-expanders.
4. *Synthesis*. We conclude that endophytic fungi have a direct effect on plant growth in a plant growth stage-dependent manner. While these effects differed between range expanders and natives, the effect strength and significance varied among the plant genera included in the present study. Nevertheless, endophytes likely influence the establishment of newly arriving plants and influence vegetation dynamics.

KEYWORDS

climate change, enemy release hypothesis, fungi, pathogens, plant–soil interactions, range-expanding plant species, root microbiome, soil biodiversity

1 | INTRODUCTION

Many studies have examined the ecology of alien plant species that have been introduced from other continents, as a small proportion of them is highly invasive, changing local biodiversity and ecosystem functioning, and causing significant economic costs (Pimentel et al., 2001; Vilà et al., 2015; Vitousek et al., 1996). However, ecological consequences of climate warming-induced range-expanding plant species have received relatively little attention (De Frenne et al., 2014; van der Putten, 2012), despite the increasing incidents of intra-continental migration of native plant species to higher altitude and latitude due to climate change (Alexander et al., 2015; van der Putten, 2012). As the growth of almost plants is influenced by the below-ground microbiome (Edwards et al., 2018; Mendes et al., 2013; Shi et al., 2016), micro-organisms may influence the success of plant range expansion as well. Especially those micro-organisms that directly penetrate plant roots have well-acknowledged positive (e.g. mycorrhizal fungi, rhizobial bacteria) and negative (e.g. bacterial, fungal and fungal-like oomycete pathogens) effects on plant growth (Raaijmakers et al., 2009). Among those players, fungi and oomycetes hold key functional roles and include major mutualists (Richardson et al., 2000; van der Heijden et al., 2015) and pathogens (Coats & Rumpho, 2014; Gilbert, 2002; Klironomos, 2002; Mendes et al., 2013; Mills & Bever, 1998).

Indeed, mutualistic micro-organisms are able to promote the success of invasive alien plants (Bever, 2002; Pringle et al., 2008) by enhancing plant abundance. Invasive alien plants even may change community composition of fungal mutualists to their own favour (Mummey & Rillig, 2006; Stinson et al., 2006; Zhang et al., 2010). In contrast to mutualists, many pathogens are limited in their host range (Gilbert & Webb, 2007) and often have a limited biogeographical distribution (Rout & Callaway, 2012). This has likely consequences for plant range shifts, as native plants can become released from their specialized pathogens, a hypothesis predicted by the enemy release hypothesis for introduced alien plants (Keane & Crawley, 2002; Mitchell & Power, 2003; Reinhart et al., 2003; Wolfe, 2002). However, pathogen effects may depend on plant growth stage, with mature plants generally suffering less from pathogens than seeds or seedlings, with profound importance especially on short-lived herbaceous plant species (Bagchi et al., 2014; Blaney & Kotanen, 2001; Gilbert, 2002; Jarosz & Davelos, 1995; Mordecai, 2015; Packer & Clay, 2000).

Similar to invaders from inter-continental origin, plants that expand their range within a continent may be less exposed to negative feedback from their soil microbiome, and host lower numbers of enemies in the new range (Dostálek et al., 2016; Morriën et al., 2010; van Grunsven et al., 2007, 2010). While experiments to decipher interactions between range-shifting plant species and soil microbiomes have focused on overall plant–soil feedback patterns (Alexander et al., 2015; De Frenne et al., 2014; Dostálek et al., 2016; Engelkes et al., 2008; van Grunsven et al., 2007), the roles of specific pathogens and mutualists have received less attention.

In general, the majority of the plant-associated microbiome components, such as diverse root-inhabiting fungi and oomycetes—in the following termed endophytes—remains functionally unknown (Arnold et al., 2000; Bamisile et al., 2018; Busby et al., 2016; Gange et al., 2019; Jumpponen & Trappe, 1998; Porras-Alfaro & Bayman, 2011; Rodriguez et al., 2009; Vandenkoornhuyse et al., 2002). Like pathogens, many endophytes might have a restricted host range especially at the plant species level (Hawksworth & Rossman, 1997; U'Ren et al., 2012, 2019). However, many endophytes might also be generalists in their host preference (Knapp et al., 2012). Overall, endophytes are suggested to be positive for plant growth, especially under stress from insect attack (Gange et al., 2019), salt stress (Gonzalez Mateu et al., 2020) or other challenging factors.

Yet, under some conditions endophytes can shift, from plant-neutral to mutualistic (Arnold et al., 2003; Clay & Holah, 1999; Clay & Schardl, 2002) or pathogenic (Busby et al., 2016; Hyde & Soytong, 2008; Kia et al., 2017; Rodriguez et al., 2009). Translating this ability to the symbiosis with invasive plants, endophytes may promote the spread of invasive plant species (Molina-Montenegro et al., 2015; Shearin et al., 2018). While endophytes have been studied in the context of inter-continental plant invasions (Klironomos, 2002; Knapp et al., 2012), only one study considered endophytes in the context of climate warming-induced range-expanding plant species. This study showed that range-expanders host different endophyte communities in the new than in the native range, while endophyte communities in congeneric natives did not differ between both ranges (Geisen et al., 2017). Yet, the functional role of endophytes in plant growth during this type of range shift remains unknown (Busby et al., 2016).

In order to study how endophytes may influence range-shifting plant species during various stages of their life history, we cultivated fungal and oomycete endophytes from roots of three range-expanding plant species and three congeneric natives. All plants were grown in northern soils from their new range (the Netherlands) and southern soil from their native range (Slovenia). We taxonomically identified all endophytes by sequencing the ITS region. Finally, we tested the effects of all cultivated root endophytes on the germination rate, seedling and plant growth of all six plant species. We tested the following general hypotheses: (1) Range-expanders will have higher germination rates and produce more biomass when inoculated with endophytes isolated from the expanded northern range soil than from the native southern range soil; we did not expect differences for native plant species, as endophytes with negative effects might not have expanded together with the range-expanders but have developed with natives. (2) The effects of endophytes are irrespective of plant growth stages, because we expected a functional conservatism of endophytes.

2 | MATERIALS AND METHODS

2.1 | Plant species

Two plant species in each of the three genera *Centaurea* (*C. stoebe* and *C. jacea*; family Asteraceae), *Geranium* (*G. pyrenaicum* and *G. molle*, family Geraniaceae) and *Tragopogon* (*T. dubius* and *T. pratensis*, family

Asteraceae) were selected for this study. The first of each plant species (*C. stoebe*, *G. pyrenaicum* and *T. dubius*) in the genera originated from south-eastern Europe and expanded its range northwards in the 20th century (named: range-expanders, tRE, Sparrius, 2014). The second of each plant species in the genera is native in Europe in both the original and the expanded new range (named: natives, tNA). More details are found in Table 1. All six herbaceous plant species co-occur in riverine habitats along the river Waal in the Netherlands, which is the southernmost branch of the river Rhine. Seeds of most plant species were obtained from a seed supplier (Cruydt-Hoeck) that collects seeds from wild plant populations, with the exception of *G. molle* that we collected ourselves (Table 1).

2.2 | Endophyte culturing experiment

To culture root-inhabiting fungi and fungal-like oomycetes (in the present study collectively named 'endophytes'), we performed a greenhouse experiment using soil collected from three independent sites in Slovenia (southern soil) and three independent sites in the Netherlands (northern soil), where all six plant species commonly occur. We decided to isolate endophytes from roots of greenhouse-grown plants rather than from the field to ensure that root endophytes were collected from all soils under the same environmental circumstances. Soil was collected from the top 3–15 cm of two sublocations in each site in the Netherlands and Slovenia. Soils from the two sublocations from each site were homogenized and sieved using a 4-mm mesh size to create three independent soil samples from both Slovenia and the Netherlands. Ten per cent of the resulting six independent soil mixes was stored in the dark at 4°C until further use. The remaining soil from the Dutch sites (Nl1-3) was combined in equal parts (1:1:1), mixed with sand (2:1) and was gamma-sterilized (20 kGy; Syngenta bv) to be used as sterile background soil. Further details on soil sampling and soil properties are described in the study by Koorem et al. (2018). The procedure of inoculating 10% of alive to 90% background soils was done to ensure that abiotic differences among soil samples were reduced to a minimum (Wilschut et al., 2019; Zhang et al., 2016).

TABLE 1 Seed origin and status of the experimental plants. All seeds were obtained from plants growing in the Netherlands

Plant species	Abbreviation	Status	Seed origin
Centaurea stoebe L.	CS	RE	Cruydt-Hoeck
Centaurea jacea L.	CJ	NA	Cruydt-Hoeck
Geranium pyrenaicum Burm. f.	GM	RE	Cruydt-Hoeck
Geranium molle L.	GP	NA	Millingerwaard natural protected area
Tragopogon dubius Scop	TD	RE	Cruydt-Hoeck
Tragopogon pratensis L.	TP	NA	Cruydt-Hoeck

Note: Cruydt-Hoeck Wildebloemenzaden (Nijeberkoop, The Netherlands) is a commercial seed supplier that collects seeds from natural populations and grows them in their own fields for seed multiplication.

Abbreviations: NA, native congeneric plant species; RE, range-expanding plant species.

Prior to germination, all plant seeds were surface-sterilized by washing seeds in 0.4% sodium hypochlorite solution for 3 min followed by rinsing with sterile distilled water (H_2O_{dest}). Seeds of all plant species were germinated on sterile glass beads in a growth cabinet at 20/10°C (day/night temperature); 16 hr light/8 hr dark conditions. Seedlings were planted individually in 0.8 L pots containing 675 g of the sterilized sandy loam soil supplemented with 75 g of one of the live soil samples. This resulted in a total of 36 pots (2 geographic ranges × 3 sites as true soil replicates from each range × 6 plant species). Individual pots were weighed two times per week and watered with sterile H_2O_{dest} to a weight of 750 g, which corresponded to a dry weight-based moisture content of ~60%. The pots were placed on a cart in a greenhouse at 16 hr light, 8 hr dark; 20°C, 15°C and 60% relative humidity. The position of the pots on the carts was randomized weekly. To increase the diversity of potentially plant life stage-dependent endophytes, another seedling of the same species and the same age was added to the same pots 2 and 4 weeks after initiating the experiment. This resulted in three plants of different age per pot.

Six weeks after initiating the experiment, shoots of all three plants per pot were cut, combined and dried at 60°C for 3 days before determining the dry weight. Roots were carefully isolated from the soil and thoroughly washed under running water before cutting into pieces of ~0.5-cm lengths. Fifty randomly selected root pieces per plant individual were placed in 2-ml centrifuge tubes filled with sterile water and stored at 4°C for 1–2 days before endophyte isolation (see below), while the remaining roots were combined and dried at 60°C for 3 days before determining the dry weight.

2.3 | Isolation and molecular characterization of endophytes

The root pieces stored in the centrifuge tubes were thoroughly washed by transferring three times to new sterile demineralized H_2O (H_2O_{dest}) in order to minimize the number of root-attached spores. Subsequently, roots were transferred to centrifuge tubes filled with 70% ethanol and incubated for 7 min under occasional mixing before final transfer and washing in a centrifuge tube containing sterile H_2O_{dest}. Root pieces were placed on sterile tissue paper to dry the surface under sterile conditions in a flow cabinet. Three individual root pieces per plant and pot were placed apart from each other in ten 10-cm Petri dish filled water agar (WA; 1.6% agar, pH 6.7, ampicillin 50 mg/L), resulting in a total of 30 root pieces per pot and a total of 360 Petri dishes. Plates were stored at 20°C in the dark. Remaining roots per pot were combined and divided into three parts.

Each part was placed on a 6-cm diameter Petri dish filled with a 1:1 mix of sterile pond water and sterile H_2O_{dest} containing dry and sterile grass leaves (*Agrostis capillaris*, 2–3 cm) for baiting zoospore forming oomycetes (Pettitt et al., 2002). The resulting 108 Petri dishes were placed at room temperature under light/dark conditions. After 4 days, the grass leaves were washed in sterile H_2O_{dest}, dried on a tissue, transferred onto WA and grown at room temperature under light/dark conditions.

All WA-containing Petri dishes were checked for growth of fungi and oomycetes 4, 6, 8 and 14 days after placing the roots on WA, whereas plates with grass leaves were checked after 1, 2, 7 and 14 days. Newly formed colonies were transferred to 6-cm Petri dishes containing WA.

The resulting cultures of endophytes were transferred to three additional media, that is, 2% malt extract agar (MEA; Difco) 0.4 × oatmeal agar (OA; Difco), 0.5 × potato carrot agar (PCA; according to Crous et al. (2009)) and 0.5 × potato dextrose agar (PDA; Oxoid). For all media, the pH was adjusted to 6.7. To reduce the number of potentially duplicated isolates, only one endophyte culture isolated from the same plant individual was kept if morphologically indistinguishable from the other isolates on all tested media. This highly conservative filtering resulted in a total of 34 distinct cultures, which were taxonomically identified by BLASTn searches of their ITS region followed by maximum likelihood analyses as detailed in Supporting Information and Section 2. Sequences are accessible at NCBI GenBank under the accession numbers MT242270–MT242299. Four cultures could not be amplified with the ITS primers used leading us to identify partial 18S rRNA gene reads to obtain information on their taxonomic identity (see Supporting Information).

Taxonomic identification revealed that 32 of the 34 cultures (94%) were fungi and two cultures were oomycetes (Table 2). Based on BLASTn searches, most of the cultures (91%) had a ≥99% sequence similarity with fungi or oomycetes existing in GenBank. However, 13% of those cultures with high similarity to sequences present in GenBank only matched with sequences not affiliated to described fungal or oomycete species but with unknown environmental sequences. For those isolates, described taxa showed a sequence similarity ≤96%. This suggests that more than 20% of all of our cultures represent species or even genera that are currently unknown or represent species with so far missing sequences in databases.

We further did an a priori functional investigation based on sequence match with the best BLASTn hits. This analysis was meant to evaluate if the endophytes were known pathogens or mutualists. We obtained this information using the best BLASTn hits and literature search on functioning of that or related species and genera. This allowed us to identify most cultures (27) as endophytes that are likely not plant pathogenic. From the remaining cultures, two most closely resembled pathogens, two potential mammal pathogens and one a saprophyte, while two could not be assigned reliably a function as they were phylogenetically too divergent from known taxa.

2.4 | Inoculation experiment

2.4.1 | Seed germination

Spores and hyphae of endophytes were extracted from well-grown cultures on malt extract agar by adding sterile H_2O_{dest} and carefully suspending endophyte material using a cell scraper. Spore suspensions were equilibrated to 1×10^6 spores or hyphal pieces per ml as counted under an inverted microscope at 400× magnification. Seeds of all targeted plants were surface-sterilized immediately before use by washing seeds in 10% sodium hypochlorite solution for 3 min (*Centaurea* and *Geranium* spp.). We applied this much harsher procedure than the one described above to ensure a complete elimination of all non-endophytic micro-organisms. Yet, tests revealed that this sterilization procedure was not eliminating all seed-attached fungi from *Tragopogon* species, so we used a harsher sterilization by pre-treating those seeds in 3% HCl for 1 min before washing seeds in 10% sodium hypochlorite solution for 5 min. Surface-sterilized seeds were rinsed with sterile H_2O_{dest}.

The germination experiment was conducted in 10-cm diameter Petri dishes filled with 0.5% H_2O agar in November 2015. Pretests revealed that *T. pratensis* did not germinate on this medium. Therefore, *T. pratensis* seeds were placed in Petri dishes (9 cm diameter) with filter paper above and below the seeds and 2 ml sterile H_2O_{dest} was added. Surface-sterilized seeds of each plant species were dipped into a spore solution of individual endophyte cultures isolated from the same plant species or individual endophyte cultures isolated from the congener species. For example, the seeds of *C. jacea* were inoculated with each endophyte culture isolated from *C. jacea* and *C. stoebe* plants, the seeds of *C. stoebe* were inoculated with the endophytes isolated from *C. jacea* and *C. stoebe* plants, and so on (Figure 1). Controls were initiated by dipping surface-sterilized seeds into sterile H_2O_{dest}. Fifteen inoculated seeds were placed in each Petri dish. Each treatment was replicated four times, resulting in a total of 296 Petri dishes ([34 cultures × 2 plant groups (RE and NA) + 6 controls] × 4 replicates). After sealing Petri dishes with Parafilm, they were incubated in a fully randomized order for 11 days at 16 hr 20°C (day) and 8 hr 15°C (night) in a controlled plant growth chamber. Seeds from all plant species started to germinate at latest 2 days after placing on agar. At the 11th day the number of germinated seeds was counted to determine the total % of germinated seeds. Roots and shoots of all germinated seeds in each of the Petri dishes were harvested. Lengths of all fresh roots was measured to determine the average root length. Then, the roots and shoots were separately dried at 60°C for 36 hr and weighed.

2.4.2 | Plant growth

We germinated surface-sterilized seedlings of all six plant species (described above) on sterile glass beads in a growth cabinet at 20/10°C; 16 hr light/8 hr dark conditions. Roots of 10-day-old seedlings of each plant species were dipped in the same spore suspensions of the respective endophytes and sterile H_2O_{dest}

TABLE 2 Fungal endophytes recovered from roots the different plant species *Tragopogon dubius* (Td), *T. pratensis ssp pratensis* (TP), *Centaurea jacea* (Cj), *C. stoebe* (Cs), *Geranium mole* (Gm) and *G. pyrenaicum* (Gp) and from soils from the Netherlands (Nl) and Slovenia (S); ID: identity. Note that more detailed descriptions of the culture including the potential function are likely biased by previous studies that often focused on agricultural settings

Endophyte	Phylogroup	GenBank accession # of highest match	% similarity with best BLASTn hit	Origin of the best BLASTn hit and those with same % similarity	Details on other BLASTn hits with similar or lower % similarity	Genus (phylogenetic placement)	Comments on genus
TdS1	Fungus	JQ964802	100	Biocontrol agent	Others with same % ID include plant-associated ones, for example, KP174676 as an endophyte in tree root	*Chaetomium*	Common endophytes with known roles for plant disease control through the production of various secondary metabolites
TdS2	Fungus	KM030576	100	Isolated from bark	Other *C. globosum* with same % ID; several potential human or mammal pathogen!	*Chaetomium*	Common endophytes with known roles for plant disease control
TpS3	Fungus	KP641158	100	Endophyte in *Pistacia vera*	Other *Acremonium* spp., for example, *A. sclerotigenum* (endophyte in *Pistacia vera*) and *A. alternate*	*Acremonium*	Mostly saprophytic but some with reported role as human pathogens
TpS4	Fungus	GQ131885	100	Soils	Other *Humicola* spp as well, mostly from soils, but can be pathogenic or endophytic	*Humicola*	*Humicola* spp. also endophytic, mycoparasitic and with suggested plant disease control
TpS5	Oomycete	AY455696	100	Unknown	Also *P. pleroticum* and also *P. minus*, for example, HQ643695; some isolated from soybean (AY590279), others from soil	*Pythium*	Mostly plant pathogens
TpS6	Fungus	AB625588	100	Soils/Endophytes	Other *Humicola* spp same ID; often from soils, but can be pathogenic or endophytic	*Humicola*	Common endophytes with known roles for plant disease control
TpN7	Fungus	KF494159	100	Tomato roots and rhizosphere	Some *P. cucumerina* Leaf Spot-causing agent	*Plectosphaerella*	Most are plant pathogens
TpN8	Fungus	KF494159	100	Tomato roots and rhizosphere	Some *P. cucumerina* Leaf Spot-causing agent	*Plectosphaerella*	Most are plant pathogens
CjS9	Fungus	EU035984	100	Dog parasite	*Phialemoniopsis curvata* (human skin pathogen) same % ID	*Phialemonium*	Mammal pathogen
CjS10	Fungus	KF494089	100	Endophytes in roots of tomato and *Sesamum indicum*	Also other *F. oxysporum* spp. same % ID; for example, KC304803 causing wilt in clementine	*Fusarium*	Most of this strain plant pathogens or endophytes
CjS11	Fungus	JF755896	100	Endophyte in *Holcus lanatus*	Similar %ID with Lasiosphaeriaceae HM007093, a root endophyte of *Stipa grandis*; next known *Cercophora coprophila* with 92% ID	Unknown genus, related to *Cercophora*	Unknown
CjS12	Fungus	KJ528986	99	Soils/Endophytes	Other *Humicola* spp. with 99% ID; some endophytic and pathogenic	*Humicola*	Common endophytes with known roles for plant disease control
CjS13	Fungus	JF508361	100	Root endophyte of *Astragalus adsurgens* Pall.	Other strains (also *Slopeiomyces* = *Gaeumannomyces*) which are root endophytes in different plants	*Gaeumannomyces*	Likely non-pathogenic with potential biocontrol, some of the genus are pathogens

(Continues)

TABLE 2 (Continued)

Endophyte	Phylogroup	GenBank accession # of highest match	% similarity with best BLASTn hit	Origin of the best BLASTn hit and those with same % similarity	Details on other BLASTn hits with similar or lower % similarity	Genus (phylogenetic placement)	Comments on genus
CsS14	Fungus	JQ435795	97	Human pathogen	below 90% ID; other *Clohesyomyces* also pathogens of plants and humans	*Clohesyomyces*	Pathogens of animals and plants; also free-living aquatic taxa
CsN15	Fungus	KP641158	100	Endophyte of *Pistacia vera*	Similar % ID with *Trichothecium roseum* EU622273	*Acremonium*	Mostly saprophytic but some with reported role as human pathogens
GpN16	Fungus	JX139038	85	Too divergent to make reliable suggestions	18S rRNA gene sequence- low phylogenetic resolution	Unknown genus; sister to family Sordariaceae	Unknown
GpS17	Fungus	KM268718	100	Endophyte of tobacco	Other *Chaetomium* spp. also similar % ID	*Chaetomium*	Common endophytes with known roles for plant disease control
GpN18	Fungus	FN397268	99	Soil	Other uncultured similar % ID that are endophytic; *Podospora* spp. ≤96% ID-sometimes fungal parasites	*Podospora*	Saprophytes
GpS19	Fungus	GQ922547	99	Unknown	Other uncultured same % ID that are endophytic or from soils or fungal parasites	*Podospora*	Saprophytes
GpS20	Fungus	EU035984	100	Dog parasite	*Phialemoniopsis curvata* (human skin pathogen) same % ID	*Phialemonium*	Mammal pathogen
GpS21	Fungus	KJ528986	99	Soils/Endophytes	Other *Humicola* spp. with 99% ID; some endophytic and pathogenic	*Humicola*	Common endophytes with known roles for plant disease control
GpS22	Fungus	KF313106	99	Soils/Endophytes	Same match with other strains, root endophytes and undescribed '*Verticillium*' spp	*Simplicillium*	Common endophytes with known roles for plant disease control
GpS23	Fungus	FJ004599	100	Soils	18S rRNA gene sequence- low phylogenetic resolution	*Penicillium*	Unknown
GmS24	Fungus	AF108480	99	Soils/Endophytes	Similar to other root endophytes	*Simplicillium*	Common endophytes with known roles for plant disease control
GmN25	Fungus	FJ536208	100	Root endophyte	And other *P. macrosponosa* same % ID; most endophytes in different roots	*Periconia*	Plant pathogens and saprophytes
GmN26	Fungus	KP230824	99	Rhizosphere soil of *Panax notoginseng*; potential endophytes	Similar % ID with *Alternaria* spp. (isolated from orchid roots)	*Pyrenochaeta*	Human and plant pathogens, endophytes
GmS27	Fungus	KJ767117	99	Soil	Several *Humicola* spp same ID; often from soils, but can be pathogenic or endophytic	*Humicola*	*Humicola* spp. also endophytic, mycoparasitic and with suggested plant disease control
GmN28	Fungus	KM247477	100	Endophyte in orchids	Next matches much lower % ID; next known *Sebacina* sp. 84% ID	Unknown genus; sister to *Lophiostoma*	

(Continues)

TABLE 2 (Continued)

Endophyte	Phylogroup	GenBank accession # of highest match	% similarity with best BLASTn hit	Origin of the best BLASTn hit and those with same % similarity	Details on other BLASTn hits with similar or lower % similarity	Genus (phylogenetic placement)	Comments on genus
GmN29	Fungus	JF508361	99	Root endophyte of *Astragalus adsurgens* Pall.	Same match with other strains (also *Slopeiomyces* = *Gaeumannomyces*) which are root endophytes in different plants	*Gaeumannomyces*	Likely non-pathogenic with potential biocontrol, some of the genus are pathogens
GmN30	Oomycete	KF861548	100	Soil/endophyte	Other *P. pleroticum* and *P. minus* spp., for example, AY590279 (soybean endophyte) same % ID	*Pythium*	Mostly plant pathogens
GmN31	Fungus	GQ924005	98	Endophyte in *Bouteloua gracilis*	Likely new genus/family/order; next best match (95% ID) with uncultured fungi (GQ924022), rest lower than 90%; best known *Lophiostoma* with 86% ID	Unknown genus; sister to *Lophiostoma*	
GmN32	Fungus	KJ689788	99	Endophyte in *Quercus suber*	Next best matches (98% ID) with endophytic *Xylaria* spp. and other uncultivated taxa	*Xylaria* or *Halorosellinia*	Saprophytes and endophytes
GmN33	Fungus	KT367526.	99	Soil/endophyte	Other endophytic/pathogenic *Saccharicola* similar % ID	*Saccharicola*	Plant pathogen
GmS34	Fungus	KF468126	100	Endophyte	Other uncultured taxa same % ID, most endophytic; *Alternaria* sp. with 99%	*Alternaria*	Plant pathogen or endophytes

that were used in the germination experiment. Seedlings were randomly placed in wells of a 140 multi-well plate pre-filled with 50 g of the same sterile soil that was used in the endophyte culturing experiment. Due to logistics reasons, for each treatment we used eight replicates for *Centaurea* and *Tragopogon* spp. and seven replicates for *Geranium* spp., resulting in a total of 552 wells. The multi-well plates were incubated in a plant growth chamber under the same conditions as described above. Plants were watered two times per week with 5 ml of sterile H_2O_{dest} in the first 2 weeks after planting and 7.5 ml in the last 2 weeks of the experiment. We conducted this experiment starting from the seedlings stage without reaching maturation to capture a phase during plant growth where plants might still acquire endophytes from their surrounding soils and where effects might affect plant growth initially. Therefore, we harvested after 25 days by clipping shoots, and carefully washing roots under running H_2O_{dest}. Shoot and root biomass was determined after drying at 60°C for 36 hr.

2.5 | Data analysis

All statistical analyses were carried out in R statistical language, version 3.6.1 (R Core Team, 2019).

2.5.1 | Endophyte culturing experiment

The effects of soil origin (south or north) and plant status (range expander: tRE or native: tNA), on plant biomass were analysed using ANOVA with plant genus (*Centaurea*, *Geranium* and *Tragopogon*), soil origin (north or south), status of the tested plant (tRE or tNA) and all their interactions as fixed factors.

2.5.2 | Inoculation experiments

The percentage of germinated seeds was calculated as $p = 100 \times (\text{number of germinated seeds} + 0.5)/(\text{total number of seeds} + 1)$ to guard against 0 or 100% values. We corrected the obtained values of percentage of germination or plant biomass using the mean values of the corresponding controls. We call this corrected values 'ratio to control'. For example, for the % seed germination, the ratio was calculated as % germination with endophyte/mean % germination in control treatment. If the resulting value is below 1, the endophytes had a negative effect; a value above 1 indicates a positive effect of the endophyte; and a value of 1 indicates that there was no effect of endophytes. As each pair of species was inoculated with a different set of endophytes, the effects of endophytes for each pair of congeners were analysed separately. The ratios were analysed using mixed-effects models. Prior to analysis, the ratios were log-transformed as ln(ratio + 0.5) to meet the assumptions of homogeneity of variances. The fixed effect structure of the full model for *Geranium*

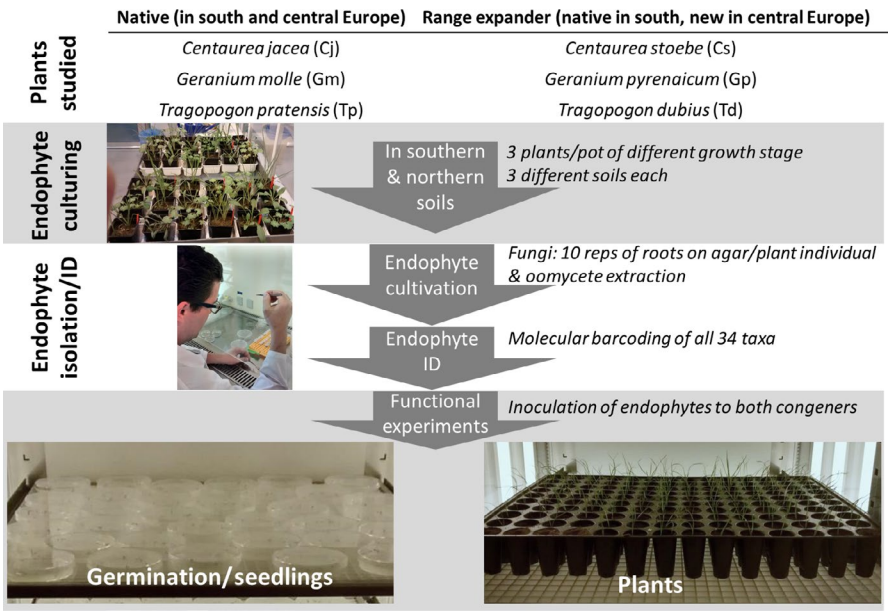

FIGURE 1 Scheme illustrating the experimental set-up. We grew three pairs of native and range-expanding plant species in soils from the range-expanders' origin (South Europe) and the expanded range (Central Europe). We then cultivated root endophytes by placing root pieces on fungal- and oomycete-specific media. We obtained 34 unique cultures that we molecularly identified and used for functional experiments to test their effect on seed germination, seedling growth and plant growth [Colour figure can be viewed at wileyonlinelibrary.com]

pair was: endophyte soil origin (eSouth or eNorth), endophyte plant origin (eRE or eNA), status of the tested plant (tRE or tNA) and all possible interactions. Because we were not able to initiate cultures from all plant species and soils, it was not possible to include endophyte soil origin by endophyte plant origin and three-way interactions in the full models for *Centaurea* and *Tragopogon* pairs. Furthermore, as only one endophyte was cultivated in the Southern soil from both *Centaurea* species, we excluded the factor endophyte soil origin from their models. Within all mixed models, Petri dish (or Well) or endophyte identity was treated as random effect to account that plants exposed to the same fungal culture in a Petri dish or Well are pseudo-replicates. Model selection was performed using the corresponding full model as a starting point. The best performing models were selected using an information theoretical approach (Akaike information criterion, AIC, Burnham & Anderson, 2004) and likelihood ratio tests. The detailed description of the model selection procedure for each congeneric pair is described in the Supporting Information. The final selected models for each congeneric pair and their numerical outputs are shown in Table S2 (in Supporting Information).

The assumption of the homogeneity of variance and normality were checked graphically by inspecting the residuals plotted against fitted values, and against each explanatory variable in or outside the model. The Cook's distance values (Cook & Weisberg, 1982) were used to detect any influential observations. Two observations from two different datasets had much smaller residuals compared to the rest of the data points. After inspecting the raw data and confirming that these data points were largely differing from the rest in the same group, the two points were deleted from the analyses.

The mixed models were fitted using *lmer* function (R LME4 package; (Bates et al., 2015)). To obtain the *p*-values presented in the results, we used Type III Wald *F* tests with Kenward-Roger correction for degrees of freedom from the CAR package (Fox & Weisberg, 2019). The factors were set to sum-to-zero contrasts (*contr. sum*), which compare each level of the factor to the average of the other levels.

In the Section 3, we only show those figures that were directly linked to our hypotheses (Hypothesis 1: Range-expanders perform best with endophytes from northern soils while natives do not show such a pattern; Hypothesis 2: endophytes affect plants in the same way throughout plant growth).

3 | RESULTS

3.1 | Endophyte culturing experiment

In total, 34 unique fungal and oomycete endophyte cultures were obtained from roots of all six plant species grown in both northern and southern soils (Table 2; Table S1). Plants grown in northern soil produced on average more total biomass than in southern soil ($F_{1,24} = 4.45$, $p = 0.045$; Figure S1a).

3.2 | Inoculation experiments

3.2.1 | Germination

Seed germination of range-expanding *Geranium* was on average more negatively affected by the inoculated endophytes than its native congener ($F_{1,36} = 27.94$; $p < 0.001$; Figure 2A). Seed germination of range-expanding and native *Centaurea* species was not affected by the endophytes ($p > 0.05$ in all cases, Table S2). Seed germination of range-expanding *Tragopogon* species was on average more negatively affected by the endophytes than its native congener, but the latter effect depended on whether the endophyte originated from the northern or southern soil (Figure 2B). Specifically, seed germination of the native *Tragopogon* was positively affected by endophytes

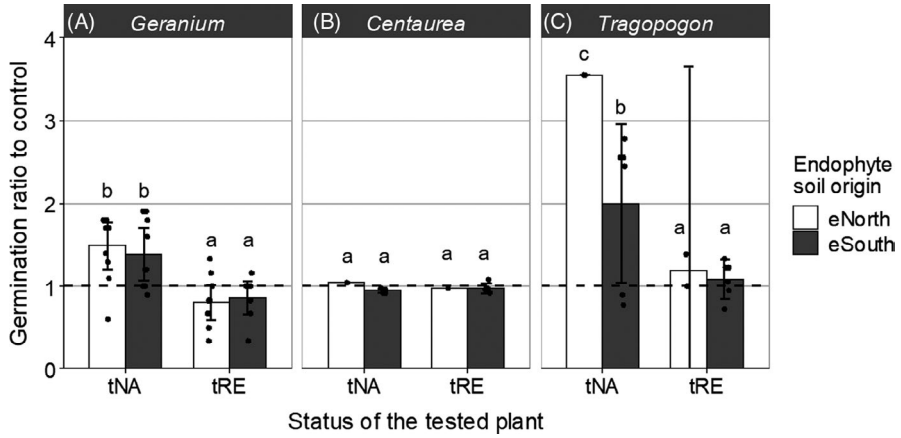

FIGURE 2 Ratio of percentage seed germination inoculated with endophytes to the averaged seed germination in control treatment. Means of the ratios ± standard errors are shown. Difference between native (tNA) and range-expanding (tRE) *Geranium* (A), *Centaurea* (B) and *Tragopogon* (C) species exposed to the endophytes originating in northern (eNorth) and southern soils (eSouth). Black solid points depicture average responses of seeds exposed to the same fungal culture. The number of endophyte cultures is given in Table S1. Different letters indicate significant differences based on linear model analysis

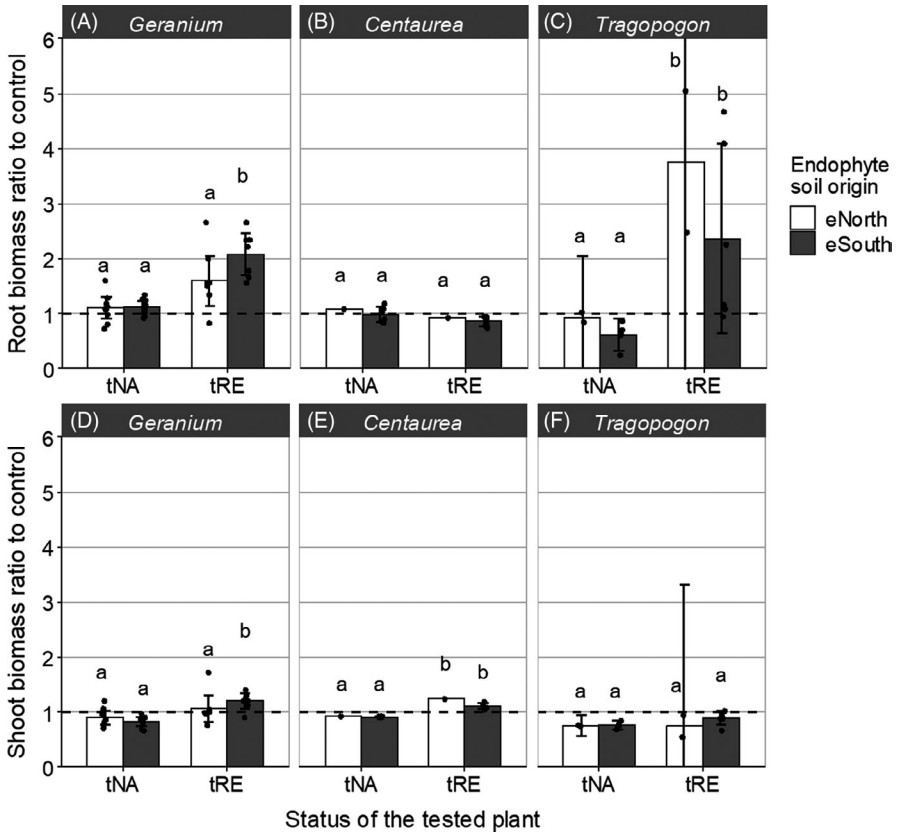

FIGURE 3 Ratio of biomass of the seedlings inoculated with endophytes to the averaged biomass in control treatment. Means of the ratios ± standard errors are shown. Difference in seedlings root (top A–C) and shoot (bottom D–F) biomass between native (tNA) and range-expanding (tRE) *Geranium* (left A and D), *Centaurea* (middle B and E) and *Tragopogon* (right C and F) species exposed to endophytes originating in northern (eNorth) and southern soils (eSouth). Black solid points depicture average responses of seedlings exposed to the same fungal culture. The number of endophyte cultures is given in Table S1. Black-dashed line indicates no effect of endophytes. Different letters indicate significant differences based on linear model analysis

retrieved from the northern soil when compared to the southern soil ($F_{1,54} = 6.53$; $p = 0.014$; Figure 2B).

3.2.2 | Seedling biomass

Range-expanding *G. pyrenaicum* seedlings produced more root biomass when exposed to the endophytes than the native *G. molle*, but this effect depended on the endophyte's soil origin ($F_{1,32} = 4.17$; $p = 0.049$, Figure 3A). Endophytes originating from southern soil promoted the root biomass of the range-expanding *G. pyrenaicum* seedlings more than the endophytes from the northern soil, whereas native *G. molle* root biomass was not affected by origin of the endophyte (Figure 3A). A similar interactive effect of endophyte soil origin and status of the tested plant was found for the shoot biomass of *Geranium* species, but this effect was marginally significant ($F_{1,32} = 4.04$; $p = 0.053$,

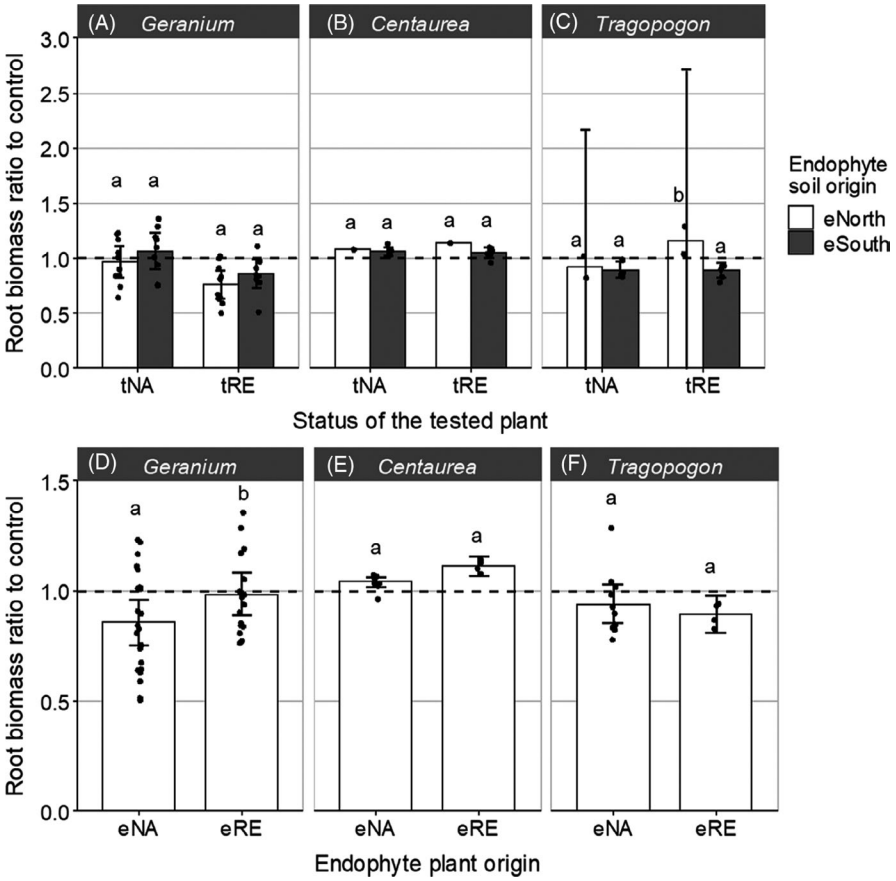

FIGURE 4 Ratio of root biomass of the plants inoculated with endophytes to the averaged root biomass in control treatment. Means of the ratios ± standard errors are shown. Top (A–C): Difference in plant root biomass of native (tNA) and range-expanding (tRE) *Geranium* (left A and D), *Centaurea* (middle B and E) and *Tragopogon* (right C and F) species exposed to endophytes originating in northern (eNorth) and southern soils (eSouth). Bottom (D–F): Difference in root biomass based on whether endophytes were isolated from native (eNA) or range-expanding (eRE) plant species. Black solid points depicture average responses of plants exposed to the same fungal culture. The number of endophyte cultures is given in Table S1. Black-dashed line indicates no effect of endophytes. Different letters indicate significant differences based on linear model analysis

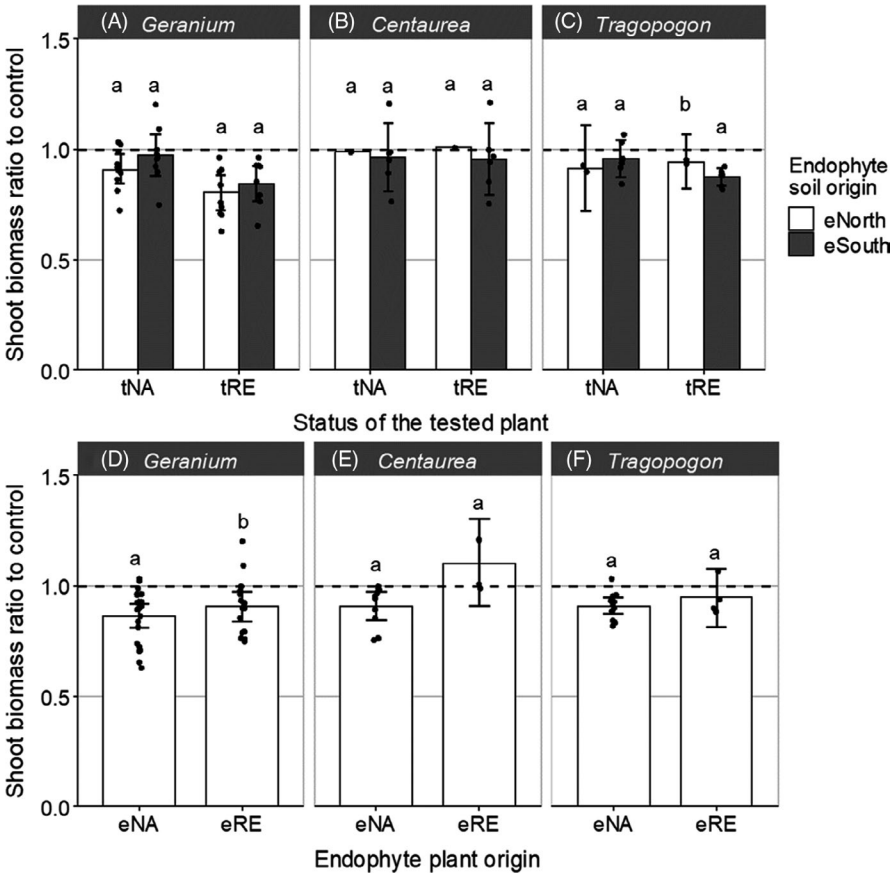

FIGURE 5 Ratio of shoot biomass of the plants inoculated with endophytes to the averaged shoot biomass in control treatment. Means of the ratios ± standard errors are shown. Top (A–C): Difference in plant shoot biomass of native (tNA) and range-expanding (tRE) *Geranium* (left A and D), *Centaurea* (middle B and E) and *Tragopogon* (right C and F) species exposed to endophytes originating in northern (eNorth) and southern soils (eSouth). Bottom (D–F): Difference in shoot biomass based on whether endophytes were isolated from native (eNA) or range-expanding (eRE) plant species. Black solid points depicture average responses of plants exposed to the same fungal culture. The number of endophyte cultures is given in Table S1. Black-dashed line indicates no effect of endophytes. Different letters indicate significant differences based on linear model analysis

Figure 3B). The root biomass of *Centaurea* seedlings was not affected by inoculated endophytes ($p > 0.05$ in all cases, Table S2), whereas shoot biomass of native *C. jacea* was negatively affected by the endophytes compared to the range-expanding congener ($F_{1,12} = 68.55$; $p < 0.001$, Figure 3C). The root biomass of native *T. pratensis* was negatively affected by the endophytes compared to the range-expanding congener ($F_{1,12} = 18.05$; $p = 0.001$, Figure 3D), whereas the shoot biomass of *Tragopogon* species was not affected by the endophytes ($p > 0.05$ in all cases, Table S2). Overall, we conclude that endophytes had variable effects on seedling growth that depended on the source of the endophyte and plan genus studied.

3.2.3 | Plant biomass

The root biomass of *Geranium* species was affected by the endophyte plant origin ($F_{1,35} = 4.72$; $p = 0.037$; Figure 4). Here, endophytes isolated from native *Geranium molle* plants more negatively affected root biomass of *Geranium* species than those isolated from range-expanding *G. pyrenaicum*. In contrast, the shoot biomass of range-expanding *Geranium pyrenaicum* was more negatively affected by the cultivated endophytes than the native congeners ($F_{1,246} = 10.22$; $p = 0.0016$; Figure 5). In particular, the root biomass of *Geranium* plants (both *Geranium* species combined) was more negatively affected by endophytes that were isolated from native plants than from range-expanders. Plant shoot and root biomass of range-expanding and native *Centaurea* species was not affected by the endophytes ($p > 0.05$ in all cases, Figures 4 and 5; Table S2). The root biomass of the range-expanding *Tragopogon* species was positively affected by endophytes retrieved from northern soil compared to endophytes from the southern soil, whereas root biomass of the native *Tragopogon* was not affected by origin of the endophytes ($F_{1,104} = 5.56$; $p = 0.02$; Figure 4). The shoot biomass of range-expanding and native *Tragopogon* species was not affected by the endophytes ($p > 0.05$ in all cases, Figure 5; Table S2).

4 | DISCUSSION

Here we show that effects on plants of fungal and oomycete root endophytes isolated from range-expanding and congeneric native plant species are variable depending on plant life stage.

4.1 | Endophytes with unknown functions dominate root cultures

We found a wide taxonomic diversity of endophytes that include fungal and oomycete species in roots of the six plant species. Many species of oomycetes are known to be notorious plant pathogens of numerous plant species and are commonly found in soils and the plants rhizosphere (Arcate et al., 2006; Geisen et al., 2015). Despite applying an oomycete-optimized isolation technique in half of the cultivation efforts (De Cock & Lévesque, 2004), only 5.8% of our unique endophyte cultures resembled oomycetes, and only 14.7% of all endophytes most closely resembled potential plant pathogens. Based solely on a priori functional classification, most microbes cultured in both range-expanding and related native plants were non-pathogenic. Further studies are needed to confirm this pattern as this assumption is based on the comparably low number of 34 cultures and assumes an equal cultivability which selects against obligate rather than facultative plant pathogens. Interestingly, those presumable pathogens based on a priori assignments often did not negatively affect plant growth. This is partly due to the fact that there is a lack of functional knowledge of endophytes in non-crop plant species suggesting that a priori functional assignment in the little studied endophytes can be misleading especially in natural plant species (Lofgren et al., 2018; Malcolm et al., 2013). A limited cultivation efficiency of endophytes prevented a thorough investigation of differences in endophyte infection between plant species or locations—a pattern that exists for some plant species (Bickford et al., 2018; Glynou et al., 2016, 2017). Yet, we believe that our rigorous cultivation approach resulting in few cultivated endophyte species is biased in the same way across treatments. Thus, the functional tests, as discussed next, are reliable but might miss some patterns that exist along the ones tested here.

4.2 | Endophytes differentially affect range-expanding and native plant species in a life stage-dependent manner

Range-expanding and congeneric native plant species differed in their responses to endophytes, but the responses strongly depended on plant growth stages. This result opposes Hypothesis 2 (endophytes affect plants in the same way throughout plant growth). The observed patterns were also more complex than assumed in Hypothesis 1 (Range-expanders perform best with endophytes from northern soils while natives do not show such a pattern), as there were no differences between range-expanders and congeneric natives in their responses to cultures obtained from northern than southern soils. All observed differences depended on plant genus, as well as on soil and plant origin of the endophytes.

The effect of endophytes on plant growth was the most pronounced in the earliest life stages: seed germination and seedling growth. These results support the idea that the impact of plant host-associated organisms depends on plant life stages, being most strong in early plant growth stages (Bagchi et al., 2014; Blaney & Kotanen, 2001; Gilbert, 2002; Jarosz & Davelos, 1995; Mordecai, 2015; Packer & Clay, 2000). Nevertheless, in many experimental studies plant performance is measured as an integration of growth from seedlings to mature plants. Indeed, differences in germination success might explain the success of invasive exotic plant species when they germinate faster than related native plant species (Hirsch et al., 2012).

Our experimental test revealed that endophytes may play a key role in affecting plants especially during early growth stages such as

during germination and seedling establishment and that these effects differ between native and range-expanding plant species. However, the effects of the endophytes on plant growth in this study are not caused by known plant pathogens, but by endophytes with an unknown a priori functioning (Arnold et al., 2000; Rodriguez et al., 2009). Functions of most of these endophytes remain largely unknown and previous studies showed contradictory effects on plant growth ranging from positive (Newsham, 2011) to negative (Kia et al., 2017; Mayerhofer et al., 2012). Our results support those observations using endophyte culture-dependent analyses. The positive effects of endophytes on plant growth might be attributed to stimulated nutrient exchange comparable to that of mycorrhizal fungi (Arnold et al., 2003; Rodriguez et al., 2009). Positive endophyte effects, however, have been suggested to occur predominantly under stress, for example through secondary metabolite production that confers resistance against herbivores (Cosme et al., 2016), while negative effects can be more common under non-stress conditions (Kia et al., 2017). Our results suggest that both positive and negative effects of endophytes on plant growth are common and depend on the partners that were interacting. As endophytes influenced plant growth during germination and seedling establishment, we conclude that endophytes are not functionally neutral, but affect plant species growth at various growth stages. Furthermore, the plant species-specific effects ranging from positive to negative may provide a mechanism of endophytes influencing plant community dynamics as shown as well for plant pathogens (Benítez et al., 2013; Sarmiento et al., 2017). Thus, our results suggest that endophytes may promote range-expanding plant species during germination, whereas native plants were promoted during germination as well as seedling establishment. Ultimately, the role of endophytes needs to be determined during the entire life history of native and range-expanding plant species in order to determine their net effects on plant community composition.

4.3 | Implications for plant range shifts and outlook

Overall, using diverse cultures of root-inhabiting fungi and oomycetes, we provide evidence that root endophytes can affect plant growth, with potential consequences for the success of some range-expanding plant species. However, a limitation of the present study is the relatively low number of pairs tested. In order to determine the generality of these results, further studies are needed with additional plant species and from a variety of ecosystems. Furthermore, despite the notion that endophytes might hardly be affected by variation among plant genotypes, future studies should include plant seeds originating from a variation of populations rather than from one as was done in the present study. Another important challenge will also be to perform studies under semi-natural and natural conditions, including the stress conditions that may occur in the field.

Functional experimental approaches such as performed here are needed to mechanistically investigate plant–microbe interactions (Inderjit & van der Putten, 2010; Reinhart & Callaway, 2006; van der Putten, 2014). Unlike Klironomos (2002), who showed that root-associated fungi affected seedlings of exotic invasive plant species less negatively than seedlings of rare native plants, we show that this trend is less pronounced among range-expanding plant species and closely related congeneric natives. Furthermore, we show that resulting effects of endophyte–plant interactions depend on plant growth stage highlighting the importance of the timing of this interaction in determining plant growth (Sikes et al., 2016). Studies that determine plant growth after a single inoculation or single sampling point might therefore uncover only part of the ecologically relevant interactions between plants and (soil) biota. However, it has to be noted that we conducted the seed germination and seedling growth analyses under artificial conditions on agar plates, while plant growth was determined in sterilized soils. Therefore, patterns observed might deviate under more complex conditions in soils. Nevertheless, we aimed at uncovering potential interactions and therefore believe that our approach provides a valid model system for this purpose (Crowther et al., 2018). Yet, the generality of our findings that individual endophytes have differential effects on range-expanders and related natives may require further testing with other plant species and environmental conditions before conclusions may be generalized.

In conclusion, our findings suggest that a wide range of endophytes can directly impact plant growth, in addition to the known, often positive effects of AMF and negative effects of pathogenic fungi. Particularly, the impact on seed germination and seedling establishment was profound and may need to be investigated as well in agricultural settings, both to improve plant growth directly, as well as to enhance plant growth under environmental stress from drought, pathogens and other factors. While the present study shows the potential for an increased availability of biotic resources for bioengineering purposes, further steps are needed in order to explore the full consequences and possible solutions offered by fungal or fungal-like endophytes.

ACKNOWLEDGEMENTS

This work was conducted with support from ERC-ADV 323020 (SPECIALS) to W.H.v.d.P. S.G. acknowledges funding from the Netherlands Organization for Scientific Research (NWO-VENI, 016.Veni.181.078). The authors thank Carolin Weser for technical assistance with seed germination and the reviewers for constructive comments to improve their manuscript. All the authors declare that there are no conflict of interests.

AUTHORS' CONTRIBUTIONS

S.G., F.C.t.H. and W.H.v.d.P. designed the study; S.G. and F.C.t.H. performed the experiment; S.G., O.K. and L.B.S. analysed the data; S.G., O.K. and W.H.v.d.P. drafted the manuscript supplemented with comments from L.B.S. and F.C.t.H.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13584.

DATA AVAILABILITY STATEMENT

Sanger sequences are accessible at NCBI GenBank under the accession numbers MT242270–MT242299. Further data associated directly with this article are available from Dryad Digital Repository https://doi.org/10.5061/dryad.jq2bvq87v (Geisen et al., 2020).

ORCID

Stefan Geisen https://orcid.org/0000-0003-0734-727X
L. Basten Snoek https://orcid.org/0000-0001-5321-2996

REFERENCES

Alexander, J. M., Diez, J. M., & Levine, J. M. (2015). Novel competitors shape species' responses to climate change. *Nature*, *525*, 515–518. https://doi.org/10.1038/nature14952

Arcate, J., Karp, M., & Nelson, E. (2006). Diversity of peronosporomycete (oomycete) communities associated with the rhizosphere of different plant species. *Microbial Ecology*, *51*, 36–50.

Arnold, A. E., Maynard, Z., Gilbert, G. S., Coley, P. D., & Kursar, T. A. (2000). Are tropical fungal endophytes hyperdiverse? *Ecology Letters*, *3*, 267–274.

Arnold, A. E., Mejía, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., & Herre, E. A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 15649–15654. https://doi.org/10.1073/pnas.2533483100

Bagchi, R., Gallery, R. E., Gripenberg, S., Gurr, S. J., Narayan, L., Addis, C. E., Freckleton, R. P., & Lewis, O. T. (2014). Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*, *506*, 85–88. https://doi.org/10.1038/nature12911

Bamisile, B. S., Dash, C. K., Akutse, K. S., Keppanan, R., & Wang, L. (2018). Fungal endophytes: Neyond herbivore management. *Frontiers in Microbiology*, *9*, 544. https://doi.org/10.3389/fmicb.2018.00544

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Dai, B., Grothendieck, G., Green, P., & Bolker, M. B. (2015). Package 'lme4'. *Convergence*, *12*, 2.

Benítez, M.-S., Hersh, M. H., Vilgalys, R., & Clark, J. S. (2013). Pathogen regulation of plant diversity via effective specialization. *Trends in Ecology & Evolution*, *28*, 705–711. https://doi.org/10.1016/j.tree.2013.09.005

Bever, J. D. (2002). Negative feedback within a mutualism: Host–specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *269*, 2595–2601.

Bickford, W. A., Goldberg, D. E., Kowalski, K. P., & Zak, D. R. (2018). Root endophytes and invasiveness: No difference between native and non-native *Phragmites* in the Great Lakes Region. *Ecosphere*, *9*, e02526.

Blaney, C. S., & Kotanen, P. M. (2001). Effects of fungal pathogens on seeds of native and exotic plants: A test using congeneric pairs. *Journal of Applied Ecology*, *38*, 1104–1113.

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods & Research*, *33*, 261–304. https://doi.org/10.1177/0049124104268644

Busby, P. E., Ridout, M., & Newcombe, G. (2016). Fungal endophytes: Modifiers of plant disease. *Plant Molecular Biology*, *90*, 645–655. https://doi.org/10.1007/s11103-015-0412-0

Clay, K., & Holah, J. (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, *285*, 1742–1744. https://doi.org/10.1126/science.285.5434.1742

Clay, K., & Schardl, C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *The American Naturalist*, *160*, S99–S127. https://doi.org/10.1086/342161

Coats, V. C., & Rumpho, M. E. (2014). The rhizosphere microbiota of plant invaders: An overview of recent advances in the microbiomics of invasive plants. *Frontiers in Microbiology*, *5*, 368. https://doi.org/10.3389/fmicb.2014.00368

Cook, R. D., & Weisberg, S. (1982). *Residuals and influence in regression*. Chapman and Hall.

Cosme, M., Lu, J., Erb, M., Stout, M. J., Franken, P., & Wurst, S. (2016). A fungal endophyte helps plants to tolerate root herbivory through changes in gibberellin and jasmonate signaling. *New Phytologist*, *211*, 1065–1076. https://doi.org/10.1111/nph.13957

Crous, P. W., Verkley, G. J., Groenewald, J. Z., & Samson, R. (2009). *Fungal biodiversity*. Centraalbureau voor Schimmelcultures.

Crowther, T. W., Boddy, L., & Maynard, D. S. (2018). The use of artificial media in fungal ecology. *Fungal Ecology*, *32*, 87–91. https://doi.org/10.1016/j.funeco.2017.10.007

De Cock, A. W. A. M., & Lévesque, C. A. (2004). New species of *Pythium* and *Phytophthora*. *Studies in Mycology*, *50*, 481–487.

De Frenne, P., Coomes, D. A., De Schrijver, A., Staelens, J., Alexander, J. M., Bernhardt-Römermann, M., Brunet, J., Chabrerie, O., Chiarucci, A., den Ouden, J., Eckstein, R. L., Graae, B. J., Gruwez, R., Hédl, R., Hermy, M., Kolb, A., Mårell, A., Mullender, S. M., Olsen, S. L., … Verheyen, K. (2014). Plant movements and climate warming: Intraspecific variation in growth responses to nonlocal soils. *New Phytologist*, *202*, 431–441. https://doi.org/10.1111/nph.12672

Dostálek, T., Münzbergová, Z., Kladivová, A., & Macel, M. (2016). Plant–soil feedback in native vs. invasive populations of a range expanding plant. *Plant and Soil*, *399*, 209–220. https://doi.org/10.1007/s11104-015-2688-x

Edwards, J. A., Santos-Medellín, C. M., Liechty, Z. S., Nguyen, B., Lurie, E., Eason, S., Phillips, G., & Sundaresan, V. (2018). Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. *PLOS Biology*, *16*, e2003862. https://doi.org/10.1371/journal.pbio.2003862

Engelkes, T., Morriën, E., Verhoeven, K. J. F., Bezemer, T. M., Biere, A., Harvey, J. A., McIntyre, L. M., Tamis, W. L. M., & van der Putten, W. H. (2008). Successful range-expanding plants experience less aboveground and below-ground enemy impact. *Nature*, *456*, 946–948. https://doi.org/10.1038/nature07474

Fox, J., & Weisberg, S. (2019). Using car functions in other functions. https://cran.r-project.org/web/packages/car/car.pdf

Gange, A. C., Koricheva, J., Currie, A. F., Jaber, L. R., & Vidal, S. (2019). Meta-analysis of the role of entomopathogenic and unspecialized fungal endophytes as plant bodyguards. *New Phytologist*, *223*, 2002–2010. https://doi.org/10.1111/nph.15859

Geisen, S., Kostenko, O., Cnossen, M. C., ten Hooven, F. C., Vres, B., & van der Putten, W. H. (2017). Seed and root endophytic fungi in a range expanding and a related plant species. *Frontiers in Microbiology*, *8*, 1645. https://doi.org/10.3389/fmicb.2017.01645

Geisen, S., ten Hooven, F. C., Kostenko, O., Snoek, L. B., & van der Putten, W. H. (2020). Data from: Fungal root-endophytes influence plants in a species-specific manner that depends on plant's growth stage. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.jq2bvq87v

Geisen, S., Tveit, A. T., Clark, I. M., Richter, A., Svenning, M. M., Bonkowski, M., & Urich, T. (2015). Metatranscriptomic census of active protists in soils. *The ISME Journal*, *9*, 2178–2190. https://doi.org/10.1038/ismej.2015.30

Gilbert, G. S. (2002). Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology*, *40*, 13–43.

Gilbert, G. S., & Webb, C. O. (2007). Phylogenetic signal in plant pathogen–host range. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 4979–4983. https://doi.org/10.1073/pnas.0607968104

Glynou, K., Ali, T., Buch, A. K., Haghi Kia, S., Ploch, S., Xia, X., Celik, A., Thines, M., & Macia-Vicente, J. G. (2016). The local environment determines the assembly of root endophytic fungi at a continental scale. *Environmental Microbiology*, *18*, 2418–2434.

Glynou, K., Ali, T., Kia, S. H., Thines, M., & Macia-Vicente, J. G. (2017). Genotypic diversity in root-endophytic fungi reflects efficient dispersal and environmental adaptation. *Molecular Ecology*, *26*, 4618–4630. https://doi.org/10.1111/mec.14231

Gonzalez Mateu, M., Baldwin, A. H., Maul, J. E., & Yarwood, S. A. (2020). Dark septate endophyte improves salt tolerance of native and invasive lineages of *Phragmites australis*. *The ISME Journal*, *14*, 1943–1954. https://doi.org/10.1038/s41396-020-0654-y

Hawksworth, D. L., & Rossman, A. Y. (1997). Where are all the undescribed fungi? *Phytopathology*, *87*, 888–891. https://doi.org/10.1094/PHYTO.1997.87.9.888

Hirsch, H., Wypior, C., von Wehrden, H., Wesche, K., Renison, D., & Hensen, I. (2012). Germination performance of native and non-native *Ulmus pumila* populations. *NeoBiota*, *15*, 53–68. https://doi.org/10.3897/neobiota.15.4057

Hyde, K. D., & Soytong, K. (2008). The fungal endophyte dilemma. *Fungal Diversity*, *33*, 163–173.

Inderjit, J., & van der Putten, W. H. (2010). Impacts of soil microbial communities on exotic plant invasions. *Trends in Ecology & Evolution*, *25*, 512–519.

Jarosz, A. M., & Davelos, A. L. (1995). Effects of disease in wild plant populations and the evolution of pathogen aggressiveness. *New Phytologist*, *129*, 371–387.

Jumpponen, A. R. I., & Trappe, J. M. (1998). Dark septate endophytes: A review of facultative biotrophic root-colonizing fungi. *New Phytologist*, *140*, 295–310. https://doi.org/10.1046/j.1469-8137.1998.00265.x

Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, *17*, 164–170.

Kia, S. H., Glynou, K., Nau, T., Thines, M., Piepenbring, M., & Macia-Vicente, J. G. (2017). Influence of phylogenetic conservatism and trait convergence on the interactions between fungal root endophytes and plants. *The ISME Journal*, *11*, 777–790. https://doi.org/10.1038/ismej.2016.140

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, *417*, 67–70. https://doi.org/10.1038/417067a

Knapp, D. G., Pintye, A., & Kovács, G. M. (2012). The dark side is not fastidious – dark septate endophytic fungi of native and invasive plants of semiarid sandy areas. *PLoS ONE*, *7*, e32570. https://doi.org/10.1371/journal.pone.0032570

Koorem, K., Kostenko, O., Snoek, L. B., Weser, C., Ramirez, K. S., Wilschut, R. A., & van der Putten, W. H. (2018). Relatedness with plant species in native community influences ecological consequences of range expansions. *Oikos*, *127*, 981–990. https://doi.org/10.1111/oik.04817

Lofgren, L. A., LeBlanc, N. R., Certano, A. K., Nachtigall, J., LaBine, K. M., Riddle, J., Broz, K., Dong, Y., Bethan, B., Kafer, C. W., & Kistler, H. C. (2018). *Fusarium graminearum*: Pathogen or endophyte of North American grasses? *New Phytologist*, *217*, 1203–1212.

Malcolm, G. M., Kuldau, G. A., Gugino, B. K., & Jiménez-Gasco, M. D. M. (2013). Hidden host plant associations of soilborne fungal pathogens: An ecological perspective. *Phytopathology*, *103*, 538–544. https://doi.org/10.1094/PHYTO-08-12-0192-LE

Mayerhofer, M. S., Kernaghan, G., & Harper, K. A. (2012). The effects of fungal root endophytes on plant growth: A meta-analysis. *Mycorrhiza*, *23*, 119–128. https://doi.org/10.1007/s00572-012-0456-9

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, *37*, 634–663.

Mills, K. E., & Bever, J. D. (1998). Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. *Ecology*, *79*, 1595–1601.

Mitchell, C. E., & Power, A. G. (2003). Release of invasive plants from fungal and viral pathogens. *Nature*, *421*, 625–627. https://doi.org/10.1038/nature01317

Molina-Montenegro, M. A., Oses, R., Torres-Díaz, C., Atala, C., Núñez, M. A., & Armas, C. (2015). Fungal endophytes associated with roots of nurse cushion species have positive effects on native and invasive beneficiary plants in an alpine ecosystem. *Perspectives in Plant Ecology, Evolution and Systematics*, *17*, 218–226. https://doi.org/10.1016/j.ppees.2015.02.003

Mordecai, E. A. (2015). Pathogen impacts on plant diversity in variable environments. *Oikos*, *124*, 414–420. https://doi.org/10.1111/oik.01328

Morriën, E., Engelkes, T., Macel, M., Meisner, A., & van der Putten, W. H. (2010). Climate change and invasion by intracontinental range-expanding exotic plants: The role of biotic interactions. *Annals of Botany*, *105*, 843–848. https://doi.org/10.1093/aob/mcq064

Mummey, D. L., & Rillig, M. C. (2006). The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. *Plant and Soil*, *288*, 81–90. https://doi.org/10.1007/s11104-006-9091-6

Newsham, K. K. (2011). A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist*, *190*, 783–793.

Packer, A., & Clay, K. (2000). Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, *404*, 278–281. https://doi.org/10.1038/35005072

Pettitt, T. R., Wakeham, A. J., Wainwright, M. F., & White, J. G. (2002). Comparison of serological, culture, and bait methods for detection of *Pythium* and *Phytophthora* zoospores in water. *Plant Pathology*, *51*, 720–727.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T., & Tsomondo, T. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, *84*, 1–20.

Porras-Alfaro, A., & Bayman, P. (2011). Hidden fungi, emergent properties: Endophytes and microbiomes. *Annual Review of Phytopathology*, *49*, 291–315.

Pringle, A., Bever, J. D., Gardes, M., Parrent, J. L., Rillig, M. C., & Klironomos, J. N. (2008). Mycorrhizal symbioses and plant invasions. *Annual Review of Ecology Evolution and Systematics*, *40*, 699–715.

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, *321*, 341–361. https://doi.org/10.1007/s11104-008-9568-6

Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*, *170*, 445–457.

Reinhart, K. O., Packer, A., van der Putten, W. H., & Clay, K. (2003). Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, *6*, 1046–1050.

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J., & Rejmánek, M. (2000). Plant invasions—the role of mutualisms. *Biological Reviews*, *75*, 65–93.

Rodriguez, R. J., White Jr., J. F., Arnold, A. E., & Redman, R. S. (2009). Fungal endophytes: Diversity and functional roles. *New Phytologist*, *182*, 314–330.

Rout, M. E., & Callaway, R. M. (2012). Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that 'everything is not everywhere'. *Annals of Botany*, *110*, 213–222.

Sarmiento, C., Zalamea, P. C., Dalling, J. W., Davis, A. S., Stump, S. M., U'Ren, J. M., & Arnold, A. E. (2017). Soilborne fungi have host affinity and host-specific effects on seed germination and survival in a lowland tropical forest. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 11458–11463. https://doi.org/10.1073/pnas.1706324114

Shearin, Z. R. C., Filipek, M., Desai, R., Bickford, W. A., Kowalski, K. P., & Clay, K. (2018). Fungal endophytes from seeds of invasive, non-native *Phragmites australis* and their potential role in germination and seedling growth. *Plant and Soil*, *422*, 183–194. https://doi.org/10.1007/s11104-017-3241-x

Shi, S., Nuccio, E. E., Shi, Z. J., He, Z., Zhou, J., & Firestone, M. K. (2016). The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages. *Ecology Letters*, *19*, 926–936. https://doi.org/10.1111/ele.12630

Sikes, B. A., Hawkes, C. V., & Fukami, T. (2016). Plant and root endophyte assembly history: Interactive effects on native and exotic plants. *Ecology*, *97*, 484–493. https://doi.org/10.1890/15-0635.1

Sparrius, L. (2014). FLORIVON. v11.5. Dutch foundation for botanical research (FLORON).

Stinson, K. A., Campbell, S. A., Powell, J. R., Wolfe, B. E., Callaway, R. M., Thelen, G. C., Hallett, S. G., Prati, D., & Klironomos, J. N. (2006). Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biology*, *4*, e140. https://doi.org/10.1371/journal.pbio.0040140

U'Ren, J. M., Lutzoni, F., Miadlikowska, J., Zimmerman, N. B., Carbone, I., May, G., & Arnold, A. E. (2019). Host availability drives distributions of fungal endophytes in the imperilled boreal realm. *Nature Ecology & Evolution*, *3*, 1430–1437. https://doi.org/10.1038/s41559-019-0975-2

U'Ren, J. M., Lutzoni, F., Miadlikowska, J., Laetsch, A. D., & Arnold, A. E. (2012). Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *American Journal of Botany*, *99*, 898–914. https://doi.org/10.3732/ajb.1100459

van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, *205*, 1406–1423.

van der Putten, W. H. (2012). Climate change, aboveground-belowground interactions, and species' range shifts. *Annual Review of Ecology Evolution and Systematics*, *43*, 365–383.

van der Putten, W. H. (2014). Introduced tree species released from negative soil biota. *New Phytologist*, *202*, 341–343.

van Grunsven, R. H. A., van der Putten, W. H., Bezemer, T. M., Tamis, W. L. M., Berendse, F., & Veenendaal, E. M. (2007). Reduced plant–soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology*, *95*, 1050–1057.

van Grunsven, R. H. A., van Der Putten, W. H., Martijn Bezemer, T., Berendse, F., & Veenendaal, E. M. (2010). Plant–soil interactions in the expansion and native range of a poleward shifting plant species. *Global Change Biology*, *16*, 380–385.

Vandenkoornhuyse, P., Baldauf, S. L., Leyval, C., Straczek, J., & Young, J. P. W. (2002). Extensive fungal diversity in plant roots. *Science*, *295*, 2051. https://doi.org/10.1126/science.295.5562.2051

Vilà, M., Rohr, R. P., Espinar, J. L., Hulme, P. E., Pergl, J., Le Roux, J. J., Schaffner, U., & Pyšek, P. (2015). Explaining the variation in impacts of non-native plants on local-scale species richness: The role of phylogenetic relatedness. *Global Ecology and Biogeography*, *24*, 139–146.

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *The American Scientist*, *84*, 468–478.

Wilschut, R. A., van der Putten, W. H., Garbeva, P., Harkes, P., Konings, W., Kulkarni, P., Martens, H., & Geisen, S. (2019). Root traits and belowground herbivores relate to plant–soil feedback variation among congeners. *Nature Communications*, *10*, 1564. https://doi.org/10.1038/s41467-019-09615-x

Wolfe, L. M. (2002). Why alien invaders succeed: Support for the escape-from-enemy hypothesis. *The American Naturalist*, *160*, 705–711. https://doi.org/10.1086/343872

Zhang, N., Van der Putten, W. H., & Veen, G. F. (2016). Effects of root decomposition on plant–soil feedback of early- and mid-successional plant species. *New Phytologist*, *212*, 220–231. https://doi.org/10.1111/nph.14007

Zhang, Q., Yang, R., Tang, J., Yang, H., Hu, S., & Chen, X. (2010). Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. *PLoS ONE*, *5*, e12380. https://doi.org/10.1371/journal.pone.0012380

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Geisen S, ten Hooven FC, Kostenko O, Snoek LB, van der Putten WH. Fungal root endophytes influence plants in a species-specific manner that depends on plant's growth stage. *J Ecol*. 2021;109:1618–1632. https://doi.org/10.1111/1365-2745.13584