Environmental stress determines the colonization and impact of an endophytic fungus on invasive knotweed

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Abstract

1. There is increasing evidence that microbes play a key role in some plant invasions. A diverse and widespread but little understood group of plant-associated microbes are the fungal root endophytes of the order Sebacinales. They are associated with exotic populations of invasive knotweed (*Reynoutria* ssp.) in Europe, but their effects on the invaders are unknown.
2. We used the recently isolated Sebacinales root endophyte S*erendipita herbamans* to experimentally inoculate invasive knotweed and study root colonisation and effects on knotweed growth under different environmental conditions. We verified the inoculation success and fungal colonisation through immunofluorescence microscopy and qPCR.
3. We found that *S. herbamans* strongly colonized invasive knotweed in low-nutrient and shade environments, but much less under drought or benign conditions. At low nutrients, the endophyte had a positive effect on plant growth, whereas the opposite was true under shaded conditions.
4. *Synthesis.* Our study demonstrates that the root endophyte *S. herbamans* has the potential to colonize invasive knotweed fine roots and impact its growth, and it could thus also play a role in natural populations. Our results also show that effects of fungal endophytes on plants can be strongly environment-dependent, and may only be visible under stressful environmental conditions.

Key words

Biological invasions, drought, fungal endophytes, Japanese knotweed, nutrient availability, plant-microbe interactions, *Reynoutria japonica*, Sebacinales, shading, stress tolerance

Introduction

Fungal endophytes are a phylogenetically diverse and widespread group of plant-associated microbes (Rodriguez et al., 2009). They can influence the growth and reproduction of individual plants, or their resistance to abiotic stress or natural enemies (Cosme et al., 2016; Kivlin et al., 2013; Mayerhofer et al., 2013; Oberhofer et al., 2014; Rho et al., 2018; Rodriguez et al., 2008). Some of the positive effects are related to the ability of endophytes to improve the nutrition of their host plants (Behie & Bidochka, 2014). There is also evidence that endophytes can influence the diversity and composition of entire plant communities (Afkhami & Strauss, 2016; Aguilar-Trigueros & Rillig, 2016; Clay & Holah, 1999; Rudgers et al., 2004; Rudgers et al., 2005) as well as their associated ecological networks (e.g. herbivores and their parasitoids; Omacini et al., 2001). However, so far our understanding of fungal endophytes is based on experiments with very few taxa, in particular the genus *Neotyphodium* and its asexual stage *Epichloë*: Other fungal systems have been hardly studied, mainly because most fungal endophytes are often difficult to cultivate and thus controlled experiments for testing their ecological functions have so far been impossible.

An important group of fungal endophytes for which this has long been true is the Serendipitaceae family in the order of Sebacinales that contains many species with broad geographic and host ranges (Garnica et al., 2016; Weiss et al., 2011). Previous experimental work has so far been largely restricted to *Serendipita indica* (*Piriformospora indica*), and it showed that *P. indica* stimulates plant growth and influences plant nutrition and tolerances to biotic and abiotic stresses (Achatz et al., 2010; Barazani et al., 2005; Gill et al., 2016; Waller et al., 2005). Our group in Tübingen recently isolated and cultivated another widespread Serendipitaceae species, *Serendipita herbamans*, which is abundant and associated with a broad range of host species and habitats in Central Europe (Riess et al., 2014).

Soil microbes can influence plant growth and stress tolerance, and these effects are to some extent host plant-specific. As a consequence, plant-microbe interactions play a role in structuring plant communities, and there is increasing evidence that they are also important in the invasion of exotic plant species (Callaway et al., 2004; Dawson & Schrama, 2016; Inderjit & van der Putten, 2010; Klironomos, 2002). In general, plant-associated microbes may have positive or negative feedbacks on plants (Bever et al., 2012; van der Putten et al., 2013). If exotic plants accumulate biota with overall more positive effects, maybe because some of the their native pathogens did not make it to the introduced range, this may give invaders an advantage over native plants (Callaway et al., 2011; Maron et al., 2014; Mitchell & Power, 2003; Reinhart et al., 2003). Alternatively, exotic plants may influence soil biota to the detriment of the native plants, e.g. through increasing abundances of their pathogens (Mangla & Callaway, 2007) or disrupting interactions with mutualists (Meinhardt & Gehring, 2012; Stinson et al., 2006).

Most previous research on plant-microbe interactions and plant invasion has focused on soil-borne microbes rather than endophytes, even though fungal endophytes are clearly abundant and diverse also in invasive plant populations (Clay et al., 2016; Shipunov et al., 2008). Besides an interesting series of studies by Aschehoug et al. (2012, 2014) who demonstrated that the leaf endophyte *Alternaria alternata* makes invasive knapweed (*Centaurea stoebe*) more competitive and allelopathic against native North American grasses, there has so far been little experimental work on fungal endophytes and invasive plants.

One of the most problematic plant invaders of temperate Europe and North America is the Japanese knotweed (*Reynoutria japonica*) and its hybrid *R.* × *bohemica*. Their aggressive growth can damage buildings and other structures, and it has huge impacts on native plant communities and ecosystems (Aguilera et al., 2010; Gerber et al., 2008; Hejda et al., 2009). Because of these ecological and economic costs, there is great interest in controlling invasive

92 knotweed, and in understanding the biological mechanisms contributing to its success.
93 Previous experimental research indicates that chemical or microbial processes belowground,
94 or their interplay, may contribute to knotweed invasion success (Murrell et al., 2011; Parepa
95 et al., 2013; Siemens & Blossey, 2007). However, the precise mechanisms and in particular
96 microbial taxa involved in these phenomena are unknown.

97 In a preliminary screening of some invasive knotweed populations around Tübingen we
98 had found a large diversity of root-associated fungi, and 40% of the studied fine-root samples
99 also harboured ITS sequences of Sebacinales fungi, including *S. herbamans* (Table S1). Thus,
100 interactions between invasive knotweed and Sebacinales appeared to be common, and we
101 were curious about the nature of the interaction between the two taxa, in particular whether
102 these hidden and little understood, but very common, fungi influenced the growth and
103 performance of invasive knotweed. We suspected that if *S. herbamans* had an effect on
104 knotweed, it would depend on environmental conditions, in particular the resource supply of
105 the plants. We tested this through a greenhouse experiment in which we inoculated Japanese
106 knotweed and its hybrid with *S. herbamans* under benign as well as drought, shading and low-
107 nutrient conditions. To confirm inoculation success and quantify fungal colonization, we used
108 immunofluorescence microscopy and qPCR. Specifically, we asked the following questions:
109 (1) Does colonization of knotweed by *S. herbamans* depend on environmental conditions? (2)
110 What effects does the endophyte have on the growth and performance of knotweed, and (3) to
111 what extent are these effects environment-dependent?
112

113 **Material and Methods**

114 *Plant material*

115 *Reynoutria japonica* and its hybrid *Reynoutria* × *bohemica* are large perennial forbs from the
116 Polygonaceae family that have become invasive in riparian and ruderal habitats in the

117 temperate regions of Europe and North America. They are clonal plants with extensive
118 rhizome networks, and in their invasive range they often form dense monoclonal stands and
119 become extremely dominant (Aguilera et al., 2010). In our experiment, we used plant material
120 from a live collection of knotweed clones that had originally been collected across seven
121 regions in Switzerland and Germany (Krebs et al., 2010) and that had been cultivated in a
122 common garden for several years. We used rhizome cuttings from 20 *R. japonica* clones and
123 13 *R.* × *bohemica* clones, with approximately ten rhizome fragments, each containing two
124 nodes and thus one intact internode, from each clone. After removal of all fine roots, the
125 rhizome fragments were surface-sterilized using the method described by (Huang et al.,
126 2014).
127
128 *Endophyte material*
129 We worked with the endophyte *Serendipita herbamans* (DSM 27534), a member of the order
130 Sebacinales, whose discovery and isolation was described in (Riess et al., 2014). Prior to the
131 experiment, we grew the endophyte for 14 days in Petri dishes with MEA medium containing
132 2% malt extract and 1.5% agar at 20°C in the dark. We then used 5 mm plugs from these
133 plates, containing media and mycelia, to inoculate 0.5 L Erlenmeyer flasks with 250 ml malt
134 extract (2.0%) liquid medium. The inoculated flasks were incubated in the dark on a rotary
135 shaker (47-52 rpm) at 20°C. After two weeks of incubation, the resulting mycelium was
136 separated from the media and washed five times with sterile distilled water.
137
138 *Experimental set-up*
139 We set up a greenhouse experiment in which we tested the effects of endophyte inoculation
140 on knotweed growth in four different environments: control, drought, low nutrients and shade.
141 Except for the control environment, all conditions were expected to be stressful for the plants.

142 We planted individual rhizome fragments 3 cm deep in 1.5 L pots filled with a 1:3 mixture of
143 low-nutrient field soil and sand (Sand- und Kieswerk Bischoff, Rottenburg, Germany). Prior
144 to planting, we measured the length and diameter of each rhizome fragment. All pots were
145 placed on individual saucers and watered as needed. After two weeks, when all aboveground
146 shoots had appeared, we inoculated half of the pots with 0.5 g of fresh *S. herbamans*
147 mycelium which were applied in small pits close to the center of the rhizomes (Fig.1A). For
148 non-inoculated plants we also created the same pits and applied a similar volume of distilled
149 water. Another two weeks after the inoculation, we started the environmental treatments. In
150 the shade treatment, the plants were covered individually with shading mesh bags that
151 reduced light levels to approximately 20%. The low-nutrient plants did not receive any
152 fertilizer throughout the experiment, whereas all others received 7:3:6 N:P:K fertilizer (b1
153 Universal-Flüssigdünger, toom Baumarkt GmbH) equivalent to 150 kg N/ha distributed
154 across 15 applications at seven-day intervals during the stress treatments. The drought plants
155 generally received only a third of the regular watering amount and, in contrast to all other
156 plants, regularly showed signs of wilting (loss of turgor). A total of 288 plants (160 *R.*
157 *japonica* and 128 *R.* x *bohemica*) were randomly assigned to the eight treatment combinations
158 (four environmental conditions, with or without endophytes), with approximately equal
159 representation of the two taxa in each treatment. Throughout the experiment, the plants were
160 grown in a climate-controlled greenhouse, in a completely randomized order, with
161 supplemental lighting at a 14:10 h light:dark cycle at 20°C/18°C.
162
163 *Data collection*
164 15 weeks after the start of the treatments, we measured leaf chlorophyll content on four leaves
165 per plant using a handheld chlorophyll meter (SPAD 502Plus, Konica Minolta, Osaka, Japan).
166 We then removed all leaves from the stem, measured their area with a LI-3100C leaf area

167 meter (LI-COR Environmental, Lincoln, Nebraska, USA) and dried the leaves and stems of
168 each plant separately at 80°C for 3 days. We used the leaf area and leaf dry mass to calculate
169 the specific leaf area (SLA) of each plant, and we combined the leaf and stem dry mass to
170 total aboveground biomass. Finally, we carefully washed the roots of each plant and took 10
171 fine root samples from different parts of the root system that we mixed and immediately
172 stored at -20°C for subsequent DNA extraction, or placed in fixing solution (0.15% (wt/vol)
173 trichloroacetic acid in 4:1 (vol/vol) ethanol/chloroform) for microscopy.
174
175 *Assessment of endophyte colonization*
176 We assessed the fungal colonization of knotweed roots qualitatively through fluorescence
177 microscopy and quantitatively through qPCR. Both analyses were done for randomly selected
178 subsets of the plants. For the microscopy, we collected roots from three pots of each treatment
179 by species combination, including both inoculated and non-inoculated samples (altogether 16
180 x 3 = 48 samples). The root samples were stained with Wheat Germ Agglutinin-Alexa Fluor
181 488 (WGA488; Thermo Fisher, Waltham MA, USA), which specifically stains fungal cell
182 walls. The staining procedure was as described in (Deshmukh et al., 2006), and the images
183 were recorded on a Leica TCS SP5 2 confocal microscope using the bright field channel and a
184 GFP filter set for detection of WGA488.
185 For the qPCR, we analysed 10 plants from each treatment by species combination, i.e. a
186 total of 80 plants, with three replicates of non-inoculated plants and seven replicates for
187 inoculated plants. We ground fine roots to a fine powder in liquid nitrogen using a sterile
188 mortar and pestle, and we used 500 mg of this material to isolate DNA using the DNAeasy
189 Plant Mini Kit (Qiagen, Hilden, Germany). The relative amounts of *S. herbamans* DNA in the
190 samples were determined through qPCR reactions with a *S. herbamans*-specific and a
191 *Reynoutria*-specific primer pair. The *S. herbamans*-specific primer pair SerhaITS binds to the

192 ITS region of the 5.8 S rDNA sequence of *S. herbamans* (SerhaITSfw199: 5'-
193 AGCCTTGTGCGGTAAAGCGA-3', SerhaITSrev199: 5'-
194 TGTATTCCGGCACCTTAACCTC-3'). The *Reynoutria*-specific primer pair FallCHS binds
195 to the genomic DNA of the Chalcone synthase gene EF090266.2 of *Fallopia japonica* (now
196 *Reynoutria japonica*) (FallCHSfwd: 5'-GGAGATGCGTGTATATTCTT-3', FallCHSrev: 5'-
197 CCAAAGATGAAGCCATGTAG-3'. The PCR primers were designed using Primer-BLAST
198 (Ye et al. 2012). For the PCR amplification we ran real-time PCRs on a Biorad CFX96
199 Thermocycler (BioRad, Hercules CA, USA) using the ABsolute SYBR Capillary Mix
200 (Thermo Fisher, Waltham MA, USA) in a final volume of 20 µl, and the following cycler
201 programmes: 95°C for 15 min followed by 45 cycles of 95°C for 15 s, 55°C for 20 s and 72°
202 C for 20 s for the FallCHS primer pair, and 95°C for 15 min followed by 45 cycles of 95°C
203 for 15 s, 60°C for 15 s and 72°C for 10 s for the Serha199 primer pair. To calculate relative
204 amounts of *S. herbamans* DNA, we used the $2^{-deltaCt}$ method (Livak & Schmittgen, 2001)
205 using the raw threshold cycle (Ct) values determined for the *S. herbamans*- and the
206 *Reynoutria*-specific primer pairs.
207
208 *Statistical analyses*
209 To test for species differences in, and effects of environmental conditions on endophyte
210 colonization, we analysed the relative *S. herbamans* densities, as determined by qPCR, with a
211 linear model that included the effects of *Reynoutria* species, environmental treatment, and
212 their interaction as fixed factors. We analysed knotweed responses to endophytes and
213 environments with regard to three variables: aboveground biomass, leaf chlorophyll content,
214 and specific leaf area. For each response variable we fitted a linear mixed model with fungal
215 inoculation, environmental treatments, knotweed species, and their interactions included as
216 fixed factors, and clone identity included as random factor. To account for possible influences

217 of initial size differences, we included the volume of the planted rhizome as a covariate in all
218 three analyses. Prior to the analyses, the biomass and specific leaf area data were log-
219 transformed to achieve homoscedasticity. All linear models were fitted with the *lmer* function
220 in the *lme4* package (Bates et al., 2015) in R (R Core Team, 2018). We used the *effects* (Fox,
221 2003) and *ggplot2* (Wickham, 2009) packages to visualize results.
222
223 **Results**
224 The experimental inoculation of knotweed plants with *Serendipita herbamans* was successful,
225 but relative colonization rates were strongly environment-dependent (Fig. 1C; main effect of
226 environmental treatment in the linear model: $F = 27.12$, $P < 0.001$). While there were hardly
227 any fungi present in the non-inoculated samples, and the average relative colonization levels
228 remained low in the inoculated control and drought treatments, colonization increased four-
229 fold and eight-fold, respectively, under shaded and low nutrient conditions (Fig. 1C). There
230 were no differences among the two *Reynoutria* species in terms of relative fungal colonisation
231 ($F < 1$ and $P > 0.5$ for species main effect and species x treatment interaction). The
232 colonization of *Reynoutria* roots by *S. herbamans* was confirmed by fluorescence
233 microscopy. In all root samples from inoculated plants we detected hyphal structures typical
234 for *S. herbamans* on root surfaces, between the outer cell layers, and inside of some cortical
235 root cells (Fig. 1B). We also observed some hyphal structures in the roots of non-inoculated
236 plants.
237 As expected, the stress treatments in our experiment strongly impacted the growth of
238 knotweed (Table 1, Fig. 2). Compared to control plants, the biomass was reduced in all three
239 stress treatments, but particularly strongly under low-nutrient conditions. There were also
240 strong treatment effects on chlorophyll content and SLA, with a particularly low chlorophyll
241 content at low nutrient availability, and the highest SLA under shaded conditions (Fig. 2).

242 There were also differences between the two knotweed taxa (Table 1). The hybrid *Reynoutria*
243 x *bohemica* was generally larger (+ 25%) and had a higher SLA (+ 5%) than *R. japonica*, and
244 its biomass was less sensitive to drought and shading than that of *R. japonica* (Fig. S1).
245 Inoculation with *Serendipita herbamans* influenced the growth of the knotweed plants,
246 but again in a strongly treatment-dependent manner, with significant fungus by treatment
247 interactions (but no fungus main effects) for aboveground biomass and chlorophyll content,
248 and a marginally significant interaction for SLA (Table 1, Fig. 2). Under low-nutrient
249 conditions, addition of the endophyte increased knotweed biomass by 15%, but decreased it
250 by 10% in the shade, or had no effect at all under drought or control conditions. Similarly,
251 endophyte inoculation increased chlorophyll content by 13% under low nutrients but
252 decreased it by 5% under shaded conditions, and had no significant effects in the other two
253 treatments. Finally, the SLA was positively affected in the shade but negatively under low-
254 nutrient conditions, with no effects under drought or control conditions.
255
256 **Discussion**
257 Plant-microbe interactions play an important role in natural ecosystems (Bever et al., 2012;
258 Klironomos, 2002; van der Putten et al., 2013). However, the ecological function of
259 endophytic microbes that live within plants is so far little understood. In this study we show
260 that the fungal root endophyte *Serendipita herbamans* can rapidly colonize invasive knotweed
261 (*Reynoutria* ssp.) and influence its growth, with detrimental effects in the shade but
262 promotion of growth under low-nutrient conditions. Our study thus demonstrates that this
263 widespread endophyte interacts with an important invasive plant, and it also highlights the
264 environment-dependency of plant-endophyte interactions.
265
266 *Endophyte colonization*

267 Compared to previous studies on plant-Sebacinales interactions, our experiment had a rather
268 realistic set-up, with fungi inoculated into a non-sterile natural soil that presumably already
269 contained a microbial community. Microscopy and qPCR confirmed that our inoculations
270 were successful and that *S. herbamans* was able to colonize knotweed plants, which confirms
271 field observations in the Tübingen area where Sebacinales including *S. herbamans* are
272 frequent endophytes of invasive knotweed populations (Table S1). This is not surprising,
273 given the broad host range of *Serendipita herbamans*, and of Sebacinales in general, which
274 also includes native Polygonaceae (Garnica et al., 2013; Riess et al., 2014). Although exotic
275 species are known to lose specialised biotic interactions, they often interact with generalist
276 enemies and mutualists in the introduced range (Mitchell & Power, 2003; Richardson et al.,
277 2007; van Kleunen et al., 2018). However, so far we do not know how novel the interaction
278 between knotweed and *S. herbamans* really is because there are no data on endophyte
279 diversity from the native East Asian range.

280 We found that the relative colonization of knotweed plants by *S. herbamans* was
281 generally much stronger under low-nutrients or shade conditions than under control or
282 drought conditions. Thus, *Reynoutria* plants appear to actively regulate their interactions with
283 *S. herbamans* in an environment-specific fashion. It is known that plants can control fungal
284 colonization, e.g. through the production of defense compounds or secondary metabolites
285 inhibiting microbial growth (Zipfel & Oldroyd, 2017), or by diverting more carbohydrates to
286 fungal symbionts (Carbonnel & Gutjahr, 2014; Martin et al., 2017). This has also been shown
287 for the closely related *S. indica* which interferes with the immune system of host plants
288 (Jacobs et al., 2011) and influences sugar concentrations in their roots (Opitz et al., 2021).
289 The functional and adaptive explanation for this is usually that plant benefits from
290 interactions with fungi are environment-dependent, and therefore plants stimulate or restrict
291 fungal access depending on these benefits. For instance, mycorrhizal colonisation is often

292 triggered by low-nutrient conditions (Bueno de Mesquita et al., 2018). We also found that
293 relative fungal colonization was highest under low-nutrient conditions, which is in line with
294 the idea that *S. herbamans* improves the nutrition of *Reynoutria* plants. It is less clear why
295 relative colonization was also increased in shaded plants because these should have been
296 mainly carbon-limited, and under such conditions plant-microbe interactions often turn
297 parasitic, as has been shown e.g. for interactions with mycorrhiza or rhizobia (Ballhorn et al.,
298 2016; Lau et al., 2012).

299 We also detected *S. herbamans* in some non-inoculated plants. The sources of this could
300 be external, e.g. fungi spores present in the potting soil, or splash dispersal from adjacent pots.
301 However, the most likely explanation seems that *S. herbamans* was already present in some of
302 the planted rhizomes. We know that some invasive knotweed populations are naturally
303 colonized by *S. herbamans*, and we therefore cannot rule out that some surface-sterilized
304 rhizomes still harboured the fungus.

305

306 *Endophyte effects on plant growth*

307 The inoculated *S. herbamans* fungi not only successfully colonized the knotweed plants in our
308 experiment, but they also significantly impacted their growth. The magnitude and direction of
309 these effects were strongly environment-dependent. Under benign or drought conditions,
310 endophyte effects on plants were small and non-significant, whereas under low-nutrient
311 conditions inoculation had strong positive effects, and under shade conditions strong negative
312 effects on knotweed performance. Similar context-dependent effects of endophytes have been
313 found in other study systems (Davitt et al., 2010; Laitinen et al., 2016; Shaffer et al., 2018).

314 Low-nutrient conditions greatly reduced knotweed biomass, and here relative *S.*
315 *herbamans* colonization was strong and the fungus increased plant growth. This suggests an
316 active promotion of endophyte access by the plants because the fungi improve plant nutrition

317 under these conditions. The observed increase of leaf chlorophyll content, which strongly
318 correlates with leaf nitrogen content (Evans, 1989), supports this idea. We know that *S.*
319 *herbamans* improves plant growth under lab conditions (Riess et al., 2014), and that the
320 closely related *Serendipita indica* can improve the nutrient acquisition and growth of many
321 plant species (Achatz et al., 2010; Barazani et al., 2005; Giauque et al., 2019; Varma et al.,
322 1999; Waller et al., 2005). Thus, it seems very likely that *S. herbamans* also improved the
323 nutrition, and as a consequence biomass growth, of invasive knotweed in our experiment.
324 Under shade conditions, the effects of *S. herbamans* were reversed, and inoculation
325 negatively affected knotweed biomass as well as leaf chlorophyll content, suggesting that
326 under these conditions the fungus indeed turned parasitic and compromised plant nutrition.
327 Similar shifts in the directions of plant-microbe interactions have been observed in other
328 studies (Ballhorn et al., 2016; Lau et al., 2012), and the likely explanation is that the typical
329 'trade logic' of mutualistic plant-microbe interactions - microbes receive photosynthates in
330 exchange for improved nutrient uptake - only works where soil nutrients are limiting, but
331 under carbon-limited shade conditions, it does not.
332 In the control and drought treatments, colonization and growth effects of endophytes
333 were very low, indicating that under these conditions the host plants limited fungi access,
334 similar to what is known from plant-mycorrhiza interactions (Averill et al., 2019; Carbonnel
335 & Gutjahr, 2014). For the drought treatment, with episodes of plant wilting, it is also possible
336 that the spread of fungi was simply limited by the lack of moisture.
337 Our results that *S. herbamans* can promote or weaken knotweed growth depending on
338 environmental context raises intriguing questions about the habitat preferences of invasive
339 knotweeds. Across their invasive range in Europe and North America, the species mostly
340 thrive in open and nutrient-rich habitats, and benefit in particular from fluctuating nutrient
341 supply (Parepa, Fischer, et al., 2013), but they rarely spread under closed canopy (Beerling,

1991; Pyšek et al., 2009). It is possible that interactions with *S. herbamans* or other microbes contribute to these habitat preferences, by facilitating nutrient uptake in open habitats but limiting knotweed under shaded conditions. Further research - in particular field experiments - is needed to test these hypotheses.

Conclusions

Our study demonstrates that the common fungal endophyte *Serendipita herbamans* can rapidly colonize fine roots of invasive knotweed and influence its growth both positively or negatively, depending on the environmental context. As *S. herbamans* is present in at least some invasive knotweed populations, the fungus could play a role in the growth and success of knotweed in some invaded habitats. However, understanding the true significance of this plant-fungus interaction requires further data, because ecological communities are of course more complex than our experimental set-up. In its natural habitat, invasive knotweed also interacts with competitors, herbivores and other enemies and mutualists, and some of these might be interacting with *S. herbamans*, too. Thus, the next step should be multi-species experiments, in the field or using mesocosm approaches, that evaluate the impact of *S. herbamans* on knotweed and other plants in a community context.

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367 **Authors' contributions**

368 SG, MP and OB conceived and designed the experiment, SG, ZL and SH carried out the
369 experiment, FW performed microscopy and qPCR analyses, MP and OB analyzed the data,
370 SG, FW, MP and OB drafted the manuscript, and all authors contributed to its revision.

371

372 **Data availability**

373 All data from our experiment will be made available through Dryad. The sequencing data
374 from the supplement are stored at GeneBank under MZ650923 - MZ651047.

375

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Table 1. Analysis of variance testing the effects of inoculation with *Serendipita herbamans*, stress treatment and knotweed species (*Reynoutria japonica* or *R.* × *bohemica*), and their interactions, on the performance of invasive knotweed. Each linear mixed model additionally included the volume of the planted rhizome as a covariate, as well as knotweed clone identity as a random variable. Significant *P*-values are in bold.

		Aboveground biomass		Chlorophyll content		Specific leaf area	
	df	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Rhizome volume	1	12.65	**<0.001**	4.32	**0.038**	0.51	0474
Fungus	1	2.11	0.146	0.19	0.661	0.01	0.933
Treatment	3	2111.23	**<0.001**	382.03	**<0.001**	894.68	**<0.001**
Species	1	13.44	**0.001**	3.02	0.089	7.51	**0.009**
Fungus x Treatment	3	2.65	**0.049**	6.56	**<0.001**	2.20	0.088
Fungus x Species	1	0.24	0.624	0.56	0.454	0.72	0.397
Treatment x Species	3	3.83	**0.010**	7.81	0.525	1.33	0.265
Fungus x Treatment x Species	3	0.31	0.817	1.16	0.324	0.29	0.829
# Observations		283		283		281	

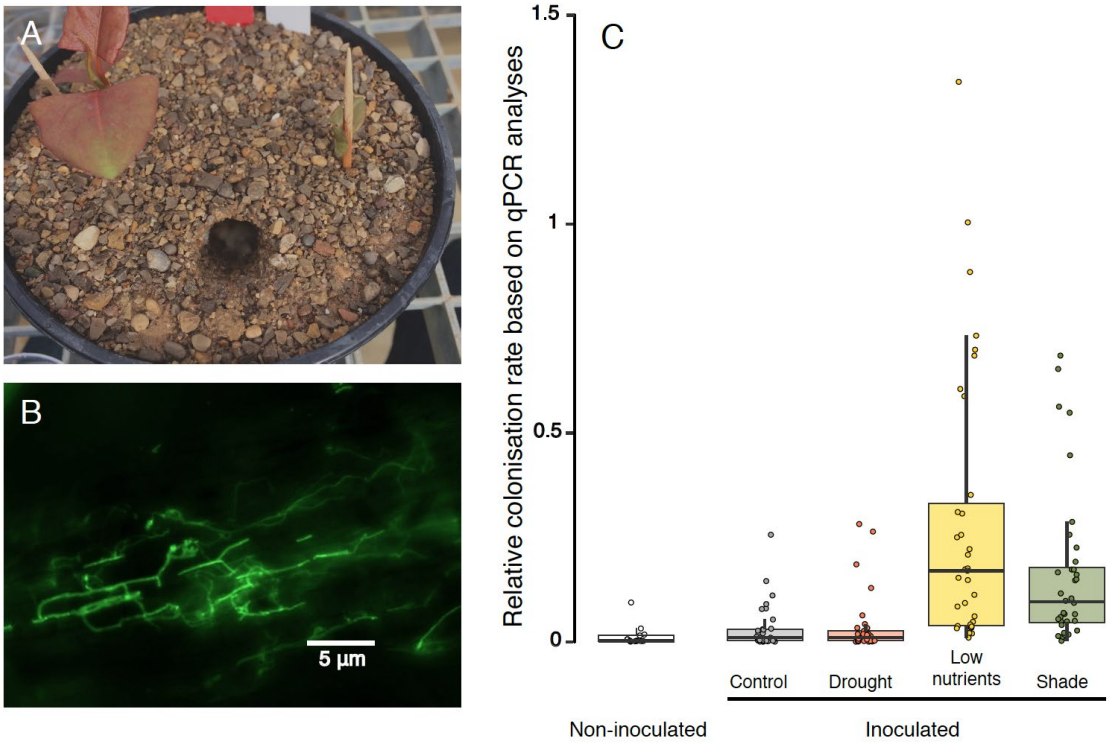

Figure 1. Experimental inoculation of *Reynoutria* plants with *Serendipita herbamans*, and the resulting fungal colonization. (A) An experimental pot right after inoculation, with freshly regenerated knotweed and the pit through which *S. herbamans* mycelium was added. (B) Fluorescence microscopic image of a root section of an inoculated *Reynoutria* plant, stained with WGA-AF 488. (C) Relative colonization rates of *S. herbamans* in the different experimental treatments, based on qPCR analyses. Points = individual observations; boxes = 25th - 75th percentiles; thick horizontal lines= medians; whiskers = 10th - 90th percentiles.

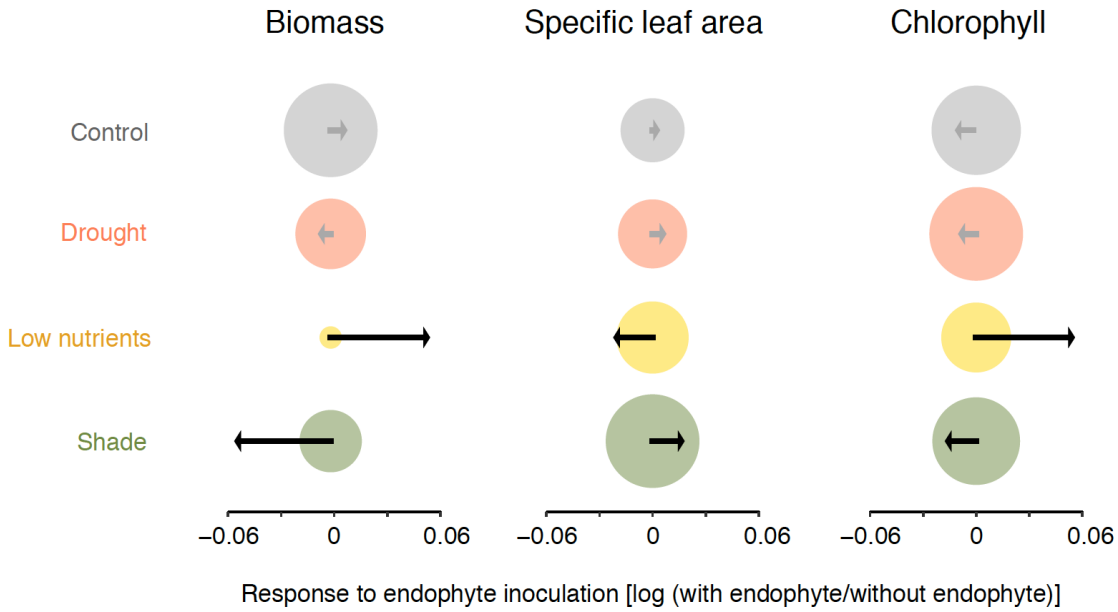

Figure 2. Effects of inoculation with *Serendipita herbamans* on invasive knotweed aboveground biomass, leaf chlorophyll content, and specific leaf area under different types of stress and in control conditions. The disc areas are proportional to the estimated means without endophyte inoculation, and the arrows show the log-responses of plants to endophyte inoculation in each treatment. Black arrows represent significant endophyte effects within a particular treatment, as indicated by Tukey's HSD test, grey arrows are non-significant.