

1 **Nematode community responses to range-expanding and native plant communities in**
2 **original and new range soils**

3 R.A. Wilschut^{1,2*}, O. Kostenko¹, K. Koorem^{1,3} & W.H. van der Putten^{1,2}

4 *Corresponding author: r.wilschut@nioo.knaw.nl

5

6 1: Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), PO

7 Box 50, 6700AB Wageningen, The Netherlands

8 2: Laboratory of Nematology, Wageningen University, PO Box 8123, 6700 ES Wageningen,

9 The Netherlands

10 3: Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, 40 Lai

11 10 St., 51005, Tartu, Estonia

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26 **Abstract**

27 Many plant species expand their range to higher latitudes in response to climate change.
28 However, it is poorly understood how biotic interactions in the new range differ from
29 interactions in the original range. Here, in a mesocosm experiment, we analyze nematode
30 community responses in original and new range soils to plant communities with either 1)
31 species native in both the original and new range, 2) range-expanding species related to these
32 natives (related range-expanders), or 3) range-expanders without native congeneric species in
33 the new range (unrelated range-expanders). We hypothesized that nematode community shifts
34 between ranges are strongest for unrelated range-expanders and minimal for plant species that
35 are native in both ranges. As a part of these community shifts, we hypothesized that range-
36 expanders, but not natives, would accumulate fewer root-feeding nematodes in their new range
37 compared to their original range. Analyses of responses of nematodes from both original and
38 new ranges and comparison between range-expanders with and without close relatives have not
39 been made before. Our study reveals that none of the plant communities experienced evident
40 nematode community shifts between the original and new range. However, in soils from the
41 new range, root-feeding nematode communities of natives and related range-expanders were
42 more similar than in soils from the original range, whereas the nematode community of
43 unrelated range-expanders was distinct from the communities of natives and related range-
44 expanders in soils from both ranges. The abundances of root-feeding nematodes were
45 comparable between the original and new range for all plant communities. Unexpectedly,
46 unrelated range-expanders overall accumulated most root-feeding nematodes, whereas related
47 range-expanders accumulated fewest. We conclude that nematode communities associated to
48 native and range-expanding plant species differ between the original and the new range, but
49 that range-expanding plant species do not accumulate fewer root-feeding nematodes in their
50 new than in their original range.

51

52 **Keywords:** range-expanding plant species, novel interactions, plant-nematode interactions,
53 plant-parasitic nematodes, root-feeding nematodes

54 **Introduction**

55 Worldwide, many native plant communities are invaded by exotic species that have been
56 introduced intentionally or unintentionally by humans (van Kleunen et al. 2015). In addition to
57 exotic species that originate from other continents, current climate change enables intra-
58 continental range expansion of plant and animal species to higher latitudes and altitudes
59 (Walther et al. 2002, Parmesan 2006). While such range-expanders have become increasingly
60 common (Tamis et al. 2005), little is known about their influence on native above- and
61 belowground plant-associated biota in their novel habitat (Van Nuland et al. 2017). The limited
62 co-evolutionary history may result in naïve responses of either plants or associated biota
63 (Verhoeven et al. 2009, Pearse et al. 2013), which makes outcomes of such novel interactions
64 difficult to predict.

65 The success of introduced exotic plant species has often been related to their possession
66 of traits that are not present in the invaded native community. Next to novel traits such as fire
67 resistance (D'Antonio and Vitousek 1992) and nitrogen fixation (Stock et al. 1995), non-native
68 plant species may also benefit from the production of metabolites that are not produced by
69 native plant species (Cappuccino and Arnason 2006). In the new range, such ‘novel weapons’
70 may suppress the growth of neighboring plant species (Callaway and Aschehoug 2000),
71 mutualists of native species (Stinson et al. 2006, Callaway et al. 2008), and natural enemies
72 (Schaffner et al. 2011, Macel et al. 2014). Because plant traits such as root chemistry are often
73 phylogenetically conserved (Agrawal et al. 2009, Pearse and Hipp 2009, Gilbert and Parker
74 2016), exotic species that are phylogenetically closely related to native flora may host more
75 natural enemies in the invaded range than distantly related range-expanders (Gilbert and Parker

76 2016). These, so-called spill-over effects of local enemies (Malmstrom et al. 2005) are
77 considered as one of the possible explanations why phylogenetically distinct exotic species can
78 become more abundant than exotic species that are strongly related to native species (Strauss
79 et al. 2006).

80 Some intra-continental range-expanders are closely related to plant species in the native
81 plant community, but are nonetheless found to be more successful in suppressing generalist
82 insects, fungal pathogens and root-feeding nematodes than their related native species
83 (Engelkes et al. 2008, Morriën et al. 2012, Morriën and van der Putten 2013, Wilschut et al.
84 2017). Range-expanders that are phylogenetically more distinct from native flora can be
85 expected to have even stronger suppressive effects on these native natural enemies, but such
86 evidence is lacking so far. Moreover, it is still largely unknown if the interactions between
87 range-expanding plant species and their natural enemies differ between their original and new
88 range as only a couple of studies (van Grunsven et al. 2010, Dostálek et al. 2015, Macel et al.
89 2017) have addressed these questions experimentally.

90 The aim of the present study was to examine plant-nematode interactions of natives,
91 range-expanders related to these natives (hereafter: related range-expanders) and range-
92 expanders without native species from the same genus in their new range (hereafter: unrelated
93 range-expanders), in soils from the new and original range. We focus on belowground plant-
94 nematode interactions, as nematodes have important roles in the soil food-web (Ferris et al.
95 2001) and can affect spatio-temporal dynamics in natural vegetation (De Deyn et al. 2003,
96 Brinkman et al. 2015). We established mesocosms with soil from either the original or the new
97 range, in which we grew communities of each of the three groups of plant species. We recorded
98 the abundance of root-feeding nematodes, as well as bacterivores, fungivores, omnivores and
99 predators in the root zones of all plant communities growing in soils from the original and the
100 new range.

101 We tested the hypotheses that 1) range-expanders, but not natives, associate with
102 different nematode communities in the original compared to the new range, mostly by
103 accumulating fewer root-feeding nematodes in soil from their new range, 2) these shifts in
104 nematode communities will be stronger for unrelated than for related range-expanders, and 3)
105 we expect that numbers of bacterivorous, fungivorous, omnivorous and predatory nematodes
106 vary less between the plant communities than root-feeding nematodes, as they are only
107 indirectly interacting with the plants (De Deyn et al. 2004, Scherber et al. 2010).

108

109 **Methods**

110 We tested our hypotheses using three types of plant communities consisting either of: 1) four
111 plant species that are native in both South-Eastern Europe, where the range-expanders originate
112 from, and North-Western Europe, where range-expanders have expanded to, 2) four plant
113 species belonging to the same genera as the natives and that have expanded their range from
114 South-Eastern Europe to North-Western Europe, or 3) four plant species that have expanded
115 their range from South-Eastern Europe to North-Western Europe and have no native species in
116 the same genus in the new range. In a greenhouse experiment, we grew all three plant
117 communities in mesocosms with a sterilized background soil, inoculated with individual
118 replicates of soil from either the original or the new range (see below). After a growth period
119 of 14 weeks, we extracted the nematode communities from the soil of each mesocosm for
120 counting and identification.

121

122 *Plant species and seed collection*

123 All plant species occur in central Netherlands in riparian habitats of the three rivers that are
124 branches of the Rhine. The majority of these plant species can be found in the same nature
125 reserves (Dutch nature observation website: <https://www.waarneming.nl>). The native plant

126 species were *Centaurea jacea* L. (Asteraceae), *Tragopogon pratensis* L. (Asteraceae),
127 *Geranium molle* L. (Geraniaceae) and *Rorippa sylvestris* (L.) Besser (Brassicaceae). As related
128 range-expanders we used *Centaurea stoebe* L., *Tragopogon dubius* Scop., *Geranium*
129 *pyrenaicum* Burm. f. and *Rorippa austriaca* Crantz. The four unrelated range-expanders were
130 *Dittrichia graveolens* (L.) Greuter (Asteraceae), *Lactuca serriola* L. (Asteraceae), *Rapistrum*
131 *rugosum* (L.) All. (Brassicaceae) and *Bunias orientalis* L. (Brassicaceae). *Centaurea stoebe*, *T.*
132 *dubius*, *R. austriaca*, *D. graveolens* and *R. rugosum* colonized the Netherlands from the 20th or
133 early 21st century onwards, while *G. pyrenaicum*, *L. serriola* and *B. orientalis* already occurred
134 in suitable habitats of the Netherlands before the 20th century, but strongly expanded their range
135 during recent decades (NDFFF 2017). Seeds of all 12 plant species originated from single, wild
136 populations growing in the Netherlands. For *G. pyrenaicum*, *T. dubius* and *T. pratensis* ssp
137 *pratensis* seeds were supplied by Cruydhoeck, a company that grows plants from field-
138 collected seeds in the Netherlands for seed production. For all other plant species we collected
139 seeds directly from plants growing in natural areas, mainly in riverine systems in eastern
140 Netherlands.

141

142 *Soil collection*

143 We collected soil from areas in Slovenia and Austria where all plant species occur naturally
144 and from the riverine system in The Netherlands where all the range-expanding plant species
145 have become established. In all three countries, we selected three riverine areas of
146 approximately 30 ha each for soil collection. The soils in all these riverine areas are comparable
147 as they all are of alpine origin. In each area, soils were collected from three sub-locations
148 separated by a distance of minimally 300 m. First we removed the upper 3 cm soil layer and
149 then collected the soil between 3 and 15 cm depth, where most living roots occur. Thereafter,
150 soil was sieved using a 4 mm mesh and gently homogenized, while keeping sub-locations

151 separate. Half the soil from each sub-location was stored at 4-8 °C, while the other half was
152 sterilized by gamma irradiation (>25 KGray) at Steris AST (Ede, The Netherlands). To compare
153 the effects of soil biota under the same abiotic conditions, we used a common sterilized
154 background soil that was a mixture of riverine sandy clay soils additionally collected from all
155 sub-locations in the Netherlands. Background soil was sieved, homogenized and then gamma-
156 sterilized as indicated above.

157

158 *Experimental set-up*

159 We first created nine soil replicates for both the original and the new range. To obtain soil
160 replicates with communities of soil organisms that represented the new and original range in a
161 general and not location-specific way, each of these nine replicate soils consisted of sterilized
162 background soil to which live soil from two sub-locations, originating from two different main
163 areas in either the original or the new range, was inoculated (see Koorem et al. 2017). This
164 approach resulted in nine soil mixes that were non-identical, yet partly overlapping in donor
165 soils, and avoided the risk of idiosyncratic differences among individual soil samples. All soils
166 were collected from sites where at least several of the plant species that were used in the
167 experiment occurred. However, to avoid that soil mixes were dominated by soil biota associated
168 with one of the focal plant species, we did not collect soil directly beneath these plant species.
169 The soil mixes representing soils from the original range were a combination of soil from one
170 of the nine Slovenian and one of the nine Austrian sub-locations (See Appendix S1). For the
171 new range, nine soil mixes were created by combining soils from two different locations in the
172 riverine system in the Netherlands (See Appendix S1), so that each sub-location was used in
173 two different soil mixes. Each mesocosm (7L, diameter 26 cm, height 20 cm) in the experiment
174 was filled with 1.5 kg of gravel (4-8 mm particles) at the bottom on top of which 4.2 kg of soil
175 was added, consisting of 80% sterilized background soil and 10% live soil inoculum from the

176 two sub-locations. To avoid potential abiotic differences between soils from the original and
177 the new ranges, we added 10% of sterilized inoculum soil from the complementary range, so
178 that in all cases every mesocosm had 10% of (sterilized or unsterilized, respectively) soil from
179 the original and 10% from the new range.

180 Per range, each of the nine soil mixes was divided over three different mesocosms,
181 resulting in 54 mesocosms (9 soil mixes \times 3 plant communities \times 2 soil origins) in total. Each
182 mesocosm was planted with two seedlings of each of the four plant species of the same plant
183 type in the Netherlands, so that on each soil mix all three plant communities were grown.
184 Seedlings were planted in a circle in a fixed order at approximately 4 cm of each other, in such
185 a way that conspecific seedlings were not close neighbours. Mesocosms were placed in a
186 climate-controlled greenhouse of 16 h 21° (day) and 8 h 16° (night) and were watered three
187 times per week in order to keep soil moisture at 60% water holding capacity. Every week, the
188 mesocosms were moved to a different position in the greenhouse in order to avoid variation
189 caused by differences in greenhouse conditions. We did not add any nutrients to the mesocosms
190 throughout the growth period. After 12 weeks of plant growth two *Mamestra brassicae* L.
191 (Lepidoptera: Noctuidae) caterpillars were introduced to pots with the soil replicates 1-5 of both
192 new and original range soils (Appendix S1) in order to test their response to the different plant
193 communities (see Koorem et al. 2017). We did not aim to test the effects of aboveground
194 herbivory on nematode community composition. The herbivory treatment was assigned to soil
195 mixes 1-5 (Appendix S1), which due to their origin likely more closely resemble each other
196 than they resemble soil mixes 6-9. For example, original range soil mixes 1-3 share soil from
197 the same sub-location in Austria, whereas mixes 7-9 all share soil from a different sub-location.
198 Because of this non-random assignment of the herbivory treatment, it is impossible to
199 disentangle herbivory effects from soil mix effects in the presented study.

200

201 *Harvest*

202 After 14 weeks of growth, shoots of all individual plants were clipped, dried at 70 °C and
203 weighed. As it was not possible to disentangle the roots of each individual plant, roots of all
204 plants were washed from the soil collectively and dried at 70 °C to constant weight. We used
205 50 g of soil (wet weight) from each pot for nematode extraction, morphological identification,
206 and counting to feeding type. Additionally, soil samples were taken for determining soil
207 moisture content, so that the number of nematodes could be expressed per dry weight of soil.
208 Nematodes were extracted from soil using an Oostenbrink elutriator (Oostenbrink 1960). After
209 extraction, we concentrated the nematode suspensions to 2 ml, after which 4 ml hot (90 °C) and
210 4 ml cold (20 °C) formaldehyde was added to fixate the nematodes before identification and
211 counting.

212

213 *Nematode identification*

214 Morphological identification and counting of nematodes was done using an inverse-light
215 microscope at 200× magnification. Per sample, all nematodes were classified to one of the five
216 feeding types (predators, root-feeders, fungivores, omnivores or bacterivores) according to
217 Yeates et al. (1993) and counted. Root-feeding nematodes were further identified to either
218 family or genus level using Bongers (1988). Root-feeding nematode genera identified were
219 *Meloidogyne* (Heteroderidae), *Paratylenchus* (Tylenchulidae), *Pratylenchus* (Pratylenchidae),
220 *Psilenchus* (Psilenchidae), and root-feeding nematode families identified were Hoplolaimidae,
221 Tylenchidae, Anguinidae, Dolichodoridae, Criconomatidae, Hemicycliophoridae and
222 Heteroderidae.

223

224 *Statistical analyses*

225 Prior to statistical analyses, soil moisture percentages were used to calculate nematode numbers
226 per 100 g dry soil. We also calculated the density of root-feeding nematode taxa per gram root,
227 as an indication of the root-feeding nematode density on plant roots. For this, we calculated
228 total number of nematodes of each taxon per mesocosm and divided those numbers by total
229 root dry weight in that mesocosm (Appendix S2; also presented in Koorem et al. 2017).

230

231 *Multivariate* analyses: First, we performed a Principal Component Analysis (PCA in Canoco
232 5; Šmilauer and Lepš 2014) comparing nematode community composition based on the
233 abundances of the five nematode feeding types. Second, in another PCA analysis, we compared
234 community composition of only the root-feeding nematode community, as root-feeding
235 nematodes were expected to show the strongest responses to plant status in the Netherlands.
236 Nematode taxa with fewer than 3 occurrences in the data set were excluded from the analyses
237 to avoid strong effects caused by rare taxa. We used the factors ‘plant community’ and ‘soil
238 origin’ to independently classify the mesocosms. In both PCA’s we included soil mix as a
239 covariate in order to account for variation between the nine soil mixes. To test the effects of
240 plant community, soil origin and their interaction on the nematode community composition we
241 used individual redundancy analyses (RDA) in Canoco 5 for both the main effects and the
242 interaction effect. The significance of the RDA-models is based on 999 Monte Carlo
243 permutations, which were restricted to incorporate the effect of soil mix.

244

245 *Univariate* analyses: All univariate analyses were performed in R version 3.1.0 (R Core
246 Development Team 2012). We selected four nematode feeding types and four root-feeding
247 nematode genera/families that - based on the PCA - contributed most to the separation of the
248 treatments. Densities of predators were so low (average 1.27 per sample) that they were not
249 modelled. We used generalized linear models with a negative binomial error distribution (Hilbe

250 2014) to model densities of the nematode feeding types in soil (N/100 g soil). Similar models
251 were used to model densities of the selected root-feeding nematode genera and families in soil
252 (N/100 g soil) and per g root (N/g root). Generalized linear models included the fixed factors
253 soil mix (nested in soil origin), plant community, soil origin and the soil origin*plant
254 community interaction. Post-hoc Wald tests were performed using the phia package (De
255 Rosario-Martinez 2013) to individually test differences between plant communities.

256

257 **Results**

258 *Nematode feeding type community composition*

259 The nematode community composition based on feeding types was significantly affected by
260 the interaction between plant community and soil origin (RDA: total variation explained:
261 22.1%; pseudo-F = 2.7, $p = 0.003$). In particular, the nematode communities accumulated by
262 related range-expanders differed between soils from the original range and soils from the new
263 range, while nematode communities accumulated by natives and unrelated range-expanders did
264 not differ between original and new range soils (Fig. 1a).

265

266 *Root-feeding nematode community composition*

267 The community composition of root-feeding nematodes was affected by the interaction between
268 plant community and soil origin (RDA: total variation explained: 21.4%; pseudo-F = 2.6, $p =$
269 0.001, Fig. 1b). In particular, all three plant communities had differently composed root-feeding
270 nematode communities. However, in the original range nematode communities of native and
271 related range-expanders were more strongly separated than in the new range. In contrast, the
272 nematode community of the unrelated range-expanders was more separated from the other
273 nematode communities in the new range compared to the old range. The root-feeding nematode

274 groups that contributed most strongly to the separation between the treatments were
275 *Meloidogyne*, *Paratylenchus*, *Hoplolaimidae* and *Tylenchidae*.

276

277 *Abundances of the nematode feeding types*

278 Differences in densities of root-feeding nematodes (N/100 g soil) were solely explained by plant
279 community type (explained deviance: 44.55, $p(X^2, df=2) < 0.0001$; Fig. 2a). Overall, unrelated
280 range-expanders accumulated more root-feeding nematodes (N/100 g soil) than natives ($X^2 =$
281 14.74, $p < 0.001$) and related range-expanders ($X^2 = 43.63$, $p < 0.0001$), whereas natives
282 accumulated more root-feeding nematodes than their related range-expanders ($X^2 = 7.69$, $p <$
283 0.01). Numbers of bacterivorous and omnivorous nematodes (N/100 g soil) differed between
284 original and new range soil: bacterivorous nematodes were most abundant in soil from the new
285 range (explained deviance: 22.32, $p(X^2, df = 1) < 0.0001$; Fig. 2b), whereas omnivorous
286 nematodes (N/100 g soil) were most abundant in soils from the original range (explained
287 deviance: 26.81, $p(X^2, df = 1) < 0.0001$; Fig. 2c). The numbers of fungivores (N/100 g soil)
288 depended on the interaction between soil origin and plant community type (explained deviance:
289 6.11, $p(X^2, df = 2) < 0.05$). In soils from the original range, fungivore densities (N/100 g soil)
290 were higher in mesocosms with unrelated range-expanders than with native plant species ($X^2 =$
291 7.13, $p < 0.01$; Fig. 2d), whereas there were no differences between plant community types in
292 soils from the new range.

293

294 *Abundances of root-feeding nematodes*

295 Responses of all root-feeding nematodes to soil origin and plant community composition
296 depended on genus/family and whether nematode numbers were analysed per 100 g soil or per
297 g root (Fig. 3). *Meloidogyne* was the most abundant root-feeder, as 44% of the root-feeding
298 nematodes in the mesocosms with natives, 30% with related and 82% with unrelated range-

299 expanders belonged to this genus. *Meloidogyne* densities were strongly affected by plant
300 community type (N/100 g soil: explained deviance: 55.15; $p(X^2, df = 2) < 0.0001$; N/g root:
301 explained deviance: 99.82; $p(X^2, df = 2) < 0.0001$; Fig. 3a, e). Densities of *Meloidogyne* in soil,
302 as well as *Meloidogyne* densities on roots, were higher in mesocosms with unrelated range-
303 expanders than with natives (N/100 g soil: $X^2 = 21.35$, $p < 0.0001$; N/g root: $X^2 = 53.33$, $p <$
304 0.001 ; Fig. 3a,e), or related range-expanders (N/100 g soil: $X^2 = 55.49$, $p < 0.0001$; N/g root:
305 $X^2 = 97.99$, $p < 0.0001$; Fig. 3a,e). *Meloidogyne* densities in mesocosms with natives were
306 higher than in mesocosms with related range-expanders (N/100 g soil: $X^2 = 8.12$, $p < 0.01$; N/g
307 root: $X^2 = 6.77$, $p < 0.01$; Fig. 3a, e).

308 Soils from the original range contained more *Hoplolaimidae* (N/100 g soil: explained
309 deviance = 13.12, $p(X^2, df = 1) < 0.001$; N/g root: explained deviance = 10.64; $p(X^2, df = 1)$
310 < 0.01 ; Fig. 3b, f) and *Tylenchidae* (N/100 g soil: explained deviance = 21.06, $p(X^2, df = 1) <$
311 0.0001 ; N/g root: explained deviance = 18.02, $p(X^2, df = 1) < 0.0001$; Fig. 3c, g) than soils from
312 the original range. The densities of *Hoplolaimidae* on roots differed also between plant
313 communities (explained deviance = 22.83, $p(X^2, df = 2) < 0.0001$; Fig. 3f): unrelated range-
314 expanders had more *Hoplolaimidae* per g root than natives ($X^2 = 10.83$; $p < 0.001$) and related
315 range-expanders ($X^2 = 18.67$, $p < 0.0001$). *Tylenchidae* densities in soil were also affected by
316 plant community type (explained deviance = 8.25, $p(X^2, df = 2) < 0.05$; Fig. 3c): both natives
317 ($X^2 = 7.02$, $p < 0.01$) and related range-expanders ($X^2 = 7.92$, $p < 0.01$) had higher *Tylenchidae*
318 densities than unrelated range-expanders. Neither plant community nor soil origin significantly
319 affected numbers of *Paratylenchus* (Fig. 3d, h).

320

321 **Discussion**

322 Climate warming-induced range-expanding plant species can experience weaker negative
323 impact in soil from the new than from the original range (van Grunsven et al. 2010, De Frenne

324 et al. 2014, Dostálek et al. 2015, Van Nuland et al. 2017). This may be caused by the loss of
325 belowground natural enemies, such as root-feeding nematodes and soil-borne pathogens, as a
326 result of plants having higher dispersal capacities than soil biota (Berg et al. 2010, Morriën et
327 al. 2010). However, biogeographic studies on soil-borne enemies along expansion gradients are
328 scarce (Van Nuland et al. 2017), and to our knowledge such studies are non-existent along intra-
329 continental latitudinal gradients. Our study shows that, differently as hypothesized, for none of
330 the plant communities there were evident differences in root-feeding nematode community
331 composition between original and new range soils, suggesting that range-expanding plant
332 species do not experience strong shifts in root-feeding nematode communities as a consequence
333 of latitudinal range expansion. Between new and original range soils, we did observe
334 differences in the community composition based on nematode feeding types, but only for
335 related range-expanders. Therefore, our hypothesis of stronger nematode community shifts
336 between the original and new range for unrelated range-expanders than for range-expanders
337 with native relatives was not confirmed.

338 Plant community effects on root-feeding nematode community composition were not
339 the same between the ranges. Most notably, in the new range the root-feeding nematode
340 community composition of unrelated range-expanders was more distinct from the communities
341 of natives and related range-expanders in the original range (Fig. 1b), suggesting distinct
342 nematode responses to these phylogenetically distant plant species in the new range. Moreover,
343 root-feeding nematode communities of natives and related range-expanders were more
344 comparable in the new range than in the original range, suggesting nematode spill-over effects
345 from natives to related range-expanders. In spite of this interactive effects between plant
346 community and soil origin on the root-feeding nematode community composition, we did not
347 find such significant interaction effects on densities of root-feeding nematodes or on root-
348 feeding nematode groups (Figs. 2 and 3, respectively). This may indicate relatively subtle shifts

349 in multiple root-feeding nematode groups that only could be detected when the full root-feeding
350 nematode community was analyzed. Densities of Hoplolaimidae and Tylenchidae were higher
351 in soils from the original than from the new range, but these effects did not depend on plant
352 community (Fig. 3) and therefore do not underlie the observed interactive effect. Also the
353 interactive effect of plant community and soil origin on the nematode community composition
354 based on nematode feeding types could not be explained by differences in the densities of
355 the different nematode feeding groups. Possibly densities of predatory nematodes play a role in
356 the statistical separation between the original and new range for the plant community of related
357 range-expanders, but total predatory nematode densities were too low to reliably model in a
358 univariate analysis.

359 The root-feeding nematode community of unrelated range-expanders differed from
360 those of native and related range-expanders. These differences in nematode community
361 composition may be explained by plant phylogeny, as the unrelated range-expanders belong to
362 different genera than the natives and related range-expanders and therefore have different traits
363 (Gilbert and Webb 2007). However, as the community of unrelated range-expanders was
364 largely dominated by annuals, whereas the other two communities include mostly perennials
365 (Koorem et al. 2017), it is also possible that nematode responses were the result of differences
366 in plant life history strategies. Annual plant species are often early-successional colonizers
367 known to develop strongly negative plant-soil feedbacks (Kardol et al. 2006), which
368 corresponds with the strong accumulation of root-feeding nematodes found in the plant
369 community of unrelated range-expanders. While the plant species in the community of
370 unrelated range-expanders had the smallest root systems (Appendix S2; Koorem et al. 2017),
371 they accumulated the highest numbers of root-feeding nematodes, suggesting poor defence
372 against nematodes. As a result, differences between plant communities were even stronger
373 when nematode densities were expressed per gram of root (Fig. 3).

374 While intercontinentally exotic early colonizers have been shown to accumulate fewer
375 natural enemies in their new than in their original range (Blumenthal et al. 2009), we found no
376 such pattern in our study. Experimental comparisons between the group of unrelated range-
377 expanders and native plant species with an annual life history strategy are needed in order to
378 examine whether there is any benefit for this group of range-expanders over ecologically
379 comparable native plant species in the new range. However, in order to examine the effects of
380 ecological novelty associated with phylogenetic distinctiveness (Strauss et al. 2006) in the
381 context of climate-driven range expansion, future studies also need to focus on unrelated range-
382 expanders with a perennial life history. Overall, our results emphasize that plant species' life
383 histories need to be taken into account when analyzing effects of biotic interactions on range-
384 expanding and exotic plant species.

385 As hypothesized, root-feeding nematodes were more clearly affected by the different
386 plant communities than the other nematode feeding groups. The community of related range-
387 expanders accumulated fewer root-feeding nematodes than their congeneric natives, which is
388 in line with a study on range-expanding plant species in their new range soil (Morriën et al.
389 2012). Our study, which considered responses of nematode communities from both the new
390 and original range, shows that range-expanders also accumulate fewer root-feeding nematodes
391 in soil from their original range than related species native in both areas of soil origin (Fig. 2).
392 These results suggest that related range-expanders on average are better defended against root-
393 feeding nematodes than related native species, regardless of the origin of the nematodes. This
394 corresponds with a previous study showing that intracontinental range-expanders were better
395 defended against an aboveground herbivore that was naïve to all of the examined plant species
396 (Engelkes et al. 2008). However, all plants used in the study by Engelkes et al. (2008), as well
397 as in the present study originated from seeds that were collected from the new range (The
398 Netherlands). We therefore cannot exclude that the strong defence against root-feeding

399 nematodes by these related range-expanders is the result of natural selection during range
400 expansion for genotypes that are especially well-defended against generalist herbivores
401 (Doorduyn and Vrieling 2011, Lin et al. 2015). Future experiments using plant populations from
402 both the original and the new ranges of the range-expanders are needed in order to examine
403 whether such shifts in plant defence traits may have occurred during climate-driven range
404 expansion (Macel et al. 2017).

405 The nematode abundances presented in our study are the net effects of bottom-up and
406 top-down control by both the plants and the micro-organisms present in the soils (Wilschut et
407 al. 2016). While bottom-up effects on nematode numbers are stronger than top-down effects,
408 potential differences between the plant communities in their ability to attract natural enemies
409 of root-feeding nematodes, such as bacteria, fungi and protists (Stirling et al. 1998, Piskiewicz
410 et al. 2007, Geisen et al. 2015) could add additional variation in root-feeding nematode
411 accumulation. Additionally, the presence of chewing herbivores on part of the replicates could
412 have had an effect on nematode numbers (Wang et al. 2017). Our experimental set-up did not
413 allow to test these effects. Nevertheless, aboveground herbivores were only present in the final
414 two weeks of the experiment, making it less likely that they had a profound effect on nematode
415 accumulation in the soils. Interestingly, plant effects strongly differed between root-feeding
416 nematode groups: while *Meloidogyne* and Hoplolaimidae densities strongly depended on the
417 plant community, such differences were not found in *Paratylenchus* and *Tylenchidae*,
418 indicating that the latter may be more generalistic and not strongly responsive to species-
419 specific plant traits, such as root chemistry (Wilschut et al. 2017). This could be due to their
420 feeding strategy (Yeates et al. 1993): while *Meloidogyne* and Hoplolaimidae partly or
421 completely feed inside the roots, *Paratylenchus* and *Tylenchidae* are ectoparasites or root-hair
422 feeders and therefore may be less affected by defence chemistry.

423 We conclude that there are no consistent shifts in nematode community composition
424 between the original and new range of range-expanding plant species, and that range-expanders
425 do not accumulate fewer root-feeding nematodes in the new range than in the original range.
426 Unexpectedly, the range-expanders without native congeners accumulated more root-feeding
427 nematodes than the natives and their congeneric related range expanders, but this might also be
428 due to their annual life-history strategy. The community of congeneric related range-expanders
429 was found to be the most suppressive to root-feeding nematodes compared to the natives, which
430 may have benefitted their range expansion. Subsequent studies are needed where plant
431 populations from both ranges will be included in the analysis, in order to elucidate the impact
432 of range-expanding plant species on native soil communities.

433

434 **Acknowledgements**

435 We thank Carolin Weser for maintenance of the mesocosm experiment, Raquel Luján Soto for
436 help with the nematode extractions and Roel Wagenaar and Henk Duyts for help with nematode
437 identification. This project was funded by ERC Advanced grant 26055290 (SPECIALS) to
438 W.H.v.d.P and by Estonian Research Council (grant PUTJD78) to K.K. This is publication
439 xxxx of the Netherlands Institute of Ecology.

440

441 **Author contributions**

442 All authors contributed to the experimental design. K.K. was responsible for the main pot
443 experiment. R.A.W. analysed the nematode communities. O.K. contributed to statistical
444 analyses. R.A.W. wrote the manuscript, with contributions of all other authors.

445

446 **Data accessibility**

447 Experimental data (nematode counts, root biomass) are uploaded to Figshare (DOI: xxxx).

449

- 450 Agrawal, A. A., M. Fishbein, R. Halitschke, A. P. Hastings, D. L. Rabosky, and S. Rasmann. 2009.
451 Evidence for adaptive radiation from a phylogenetic study of plant defenses. *Proceedings of*
452 *the National Academy of Sciences* **106**:18067-18072.
- 453 Berg, M. P., E. T. Kiers, G. Driessen, M. van der Heijden, B. W. Kooi, F. Kuenen, M. Liefjing, H. A.
454 Verhoef, and J. Ellers. 2010. Adapt or disperse: understanding species persistence in a
455 changing world. *Global Change Biology* **16**:587-598.
- 456 Blumenthal, D., C. E. Mitchell, P. Pyšek, and V. Jarošík. 2009. Synergy between pathogen release and
457 resource availability in plant invasion. *Proceedings of the National Academy of Sciences*
458 **106**:7899-7904.
- 459 Brinkman, E., H. Duyts, G. Karssen, C. Van der Stoel, and W. H. Van der Putten. 2015. Plant-feeding
460 nematodes in coastal sand dunes: occurrence, host specificity and effects on plant growth.
461 *Plant and Soil* **397**:17-30.
- 462 Callaway, R. M., and E. T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: a
463 mechanism for exotic invasion. *Science* **290**:521-523.
- 464 Callaway, R. M., D. Cipollini, K. Barto, G. C. Thelen, S. G. Hallett, D. Prati, K. Stinson, and J. Klironomos.
465 2008. Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its
466 native Europe. *Ecology* **89**:1043-1055.
- 467 Cappuccino, N., and J. T. Arnason. 2006. Novel chemistry of invasive exotic plants. *Biology Letters*
468 **2**:189-193.
- 469 D'Antonio, C. M., and P. M. Vitousek. 1992. Biological Invasions by Exotic Grasses, the Grass/Fire
470 Cycle, and Global Change. *Annual Review of Ecology and Systematics* **23**:63-87.
- 471 De Deyn, G. B., C. E. Raaijmakers, J. Van Ruijven, F. Berendse, and W. H. Van Der Putten. 2004. Plant
472 species identity and diversity effects on different trophic levels of nematodes in the soil food
473 web. *Oikos* **106**:576-586.
- 474 De Deyn, G. B., C. E. Raaijmakers, H. R. Zoomer, M. P. Berg, P. C. de Ruiter, H. A. Verhoef, T. M.
475 Bezemer, and W. H. van der Putten. 2003. Soil invertebrate fauna enhances grassland
476 succession and diversity. *Nature* **422**:711-713.
- 477 De Frenne, P., D. A. Coomes, A. De Schrijver, J. Staelens, J. M. Alexander, M. Bernhardt-Römermann,
478 J. Brunet, O. Chabrerie, A. Chiarucci, J. den Ouden, R. L. Eckstein, B. J. Graae, R. Gruwez, R.
479 Hédli, M. Hermy, A. Kolb, A. Mårell, S. M. Mullender, S. L. Olsen, A. Orczewska, G. Peterken, P.
480 Petřík, J. Plue, W. D. Simonson, C. V. Tomescu, P. Vangansbeke, G. Verstraeten, L. Vesterdal,
481 M. Wulf, and K. Verheyen. 2014. Plant movements and climate warming: intraspecific
482 variation in growth responses to nonlocal soils. *New Phytologist* **202**:431-441.
- 483 Doorduyn, L. J., and K. Vrieling. 2011. A review of the phytochemical support for the shifting defence
484 hypothesis. *Phytochemistry Reviews* **10**:99-106.
- 485 Dostálek, T., Z. Münzbergová, A. Kladvivová, and M. Macel. 2015. Plant–soil feedback in native vs.
486 invasive populations of a range expanding plant. *Plant and Soil* **399**:1-12.
- 487 Engelkes, T., E. Morrien, K. J. F. Verhoeven, T. M. Bezemer, A. Biere, J. A. Harvey, L. M. McIntyre, W.
488 L. M. Tamis, and W. H. van der Putten. 2008. Successful range-expanding plants experience
489 less above-ground and below-ground enemy impact. *Nature* **456**:946-948.
- 490 Ferris, H., T. Bongers, and R. De Goede. 2001. A framework for soil food web diagnostics: extension
491 of the nematode faunal analysis concept. *Applied Soil Ecology* **18**:13-29.
- 492 Geisen, S., J. Rosengarten, R. Koller, C. Mulder, T. Urich, and M. Bonkowski. 2015. Pack hunting by a
493 common soil amoeba on nematodes. *Environmental microbiology* **17**:4538-4546.
- 494 Gilbert, G. S., and I. M. Parker. 2016. The Evolutionary Ecology of Plant Disease: A Phylogenetic
495 Perspective. *Annual Review of Phytopathology* **54**:549-578.
- 496 Gilbert, G. S., and C. O. Webb. 2007. Phylogenetic signal in plant pathogen–host range. *Proceedings*
497 *of the National Academy of Sciences* **104**:4979-4983.

498 Hilbe, J. M. 2014. Modeling Count Data. Cambridge University Press.
499 Kardol, P., T. M. Bezemer, and W. H. van der Putten. 2006. Temporal variation in plant–soil feedback
500 controls succession. *Ecology Letters* **9**:1080-1088.
501 Koorem, K., O. Kostenko, L. B. Snoek, C. Weser, K. S. Ramirez, R. A. Wilschut, and W. H. van der
502 Putten. 2017. Relatedness with plant species in native community influences ecological
503 consequences of range expansions. *Oikos*:in press.
504 Lin, T., P. G. L. Klinkhamer, and K. Vrieling. 2015. Parallel evolution in an invasive plant: effect of
505 herbivores on competitive ability and regrowth of *Jacobaea vulgaris*. *Ecology Letters* **18**:668-
506 676.
507 Macel, M., R. C. H. de Vos, J. J. Jansen, W. H. van der Putten, and N. M. van Dam. 2014. Novel
508 chemistry of invasive plants: exotic species have more unique metabolomic profiles than
509 native congeners. *Ecology and Evolution* **4**:2777-2786.
510 Macel, M., T. Dostálek, S. Esch, A. Bucharová, N. M. van Dam, K. Tielbörger, K. J. F. Verhoeven, and Z.
511 Münzbergová. 2017. Evolutionary responses to climate change in a range expanding plant.
512 *Oecologia* **184**:543-554.
513 Malmstrom, C. M., A. J. McCullough, H. A. Johnson, L. A. Newton, and E. T. Borer. 2005. Invasive
514 annual grasses indirectly increase virus incidence in California native perennial bunchgrasses.
515 *Oecologia* **145**:153-164.
516 Morriën, E., H. Duyts, and W. H. Van der Putten. 2012. Effects of native and exotic range-expanding
517 plant species on taxonomic and functional composition of nematodes in the soil food web.
518 *Oikos* **121**:181-190.
519 Morriën, E., T. Engelkes, M. Macel, A. Meisner, and W. H. Van der Putten. 2010. Climate change and
520 invasion by intracontinental range-expanding exotic plants: the role of biotic interactions.
521 *Annals of Botany* **105**:843-848.
522 Morriën, E., and W. H. van der Putten. 2013. Soil microbial community structure of range-expanding
523 plant species differs from co-occurring natives. *Journal of Ecology* **101**:1093-1102.
524 NDFD. 2017. Verspreidingsatlas planten.
525 Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review*
526 *of Ecology Evolution and Systematics* **37**:637-669.
527 Pearse, I. S., D. J. Harris, R. Karban, and A. Sih. 2013. Predicting novel herbivore-plant interactions.
528 *Oikos* **122**:1554-1564.
529 Pearse, I. S., and A. L. Hipp. 2009. Phylogenetic and trait similarity to a native species predict
530 herbivory on non-native oaks. *Proceedings of the National Academy of Sciences* **106**:18097-
531 18102.
532 Piskiewicz, A. M., H. Duyts, M. P. Berg, S. R. Costa, and W. H. van der Putten. 2007. Soil
533 microorganisms control plant ectoparasitic nematodes in natural coastal foredunes.
534 *Oecologia* **152**:505-514.
535 Schaffner, U., W. M. Ridenour, V. C. Wolf, T. Bassett, C. Mueller, H. Mueller-Schaerer, S. Sutherland,
536 C. J. Lortie, and R. M. Callaway. 2011. Plant invasions, generalist herbivores, and novel
537 defense weapons. *Ecology* **92**:829-835.
538 Scherber, C., N. Eisenhauer, W. W. Weisser, B. Schmid, W. Voigt, M. Fischer, E.-D. Schulze, C. Roscher,
539 A. Weigelt, and E. Allan. 2010. Bottom-up effects of plant diversity on multitrophic
540 interactions in a biodiversity experiment. *Nature* **468**:553-556.
541 Stinson, K. A., S. A. Campbell, J. R. Powell, B. E. Wolfe, R. M. Callaway, G. C. Thelen, S. G. Hallett, D.
542 Prati, and J. N. Klironomos. 2006. Invasive plant suppresses the growth of native tree
543 seedlings by disrupting belowground mutualisms. *PLoS Biol* **4**:e140.
544 Stirling, G. R., L. J. Smith, K. A. Licastro, and L. M. Eden. 1998. Control of Root-knot Nematode with
545 Formulations of the Nematode-Trapping Fungus *Arthrobotrys dactyloides*. *Biological control*
546 **11**:224-230.
547 Stock, W. D., K. T. Wienand, and A. C. Baker. 1995. Impacts of invading N₂-fixing *Acacia* species on
548 patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies
549 and 15N natural abundance values. *Oecologia* **101**:375-382.

- 550 Strauss, S. Y., C. O. Webb, and N. Salamin. 2006. Exotic taxa less related to native species are more
551 invasive. *Proceedings of the National Academy of Sciences of the United States of America*
552 **103**:5841-5845.
- 553 Tamis, W. M., M. t. Zelfde, R. Meijden, and H. U. Haes. 2005. Changes in Vascular Plant Biodiversity in
554 the Netherlands in the 20th Century Explained by their Climatic and other Environmental
555 Characteristics. *Climatic Change* **72**:37-56.
- 556 van Grunsven, R. H. A., W. H. van der Putten, T. M. Bezemer, F. Berendse, and E. M. Veenendaal.
557 2010. Plant-soil interactions in the expansion and native range of a poleward shifting plant
558 species. *Global Change Biology* **16**:380-385.
- 559 van Kleunen, M., W. Dawson, F. Essl, J. Pergl, M. Winter, E. Weber, H. Kreft, P. Weigelt, J. Kartesz, M.
560 Nishino, L. A. Antonova, J. F. Barcelona, F. J. Cabezas, D. Cardenas, J. Cardenas-Toro, N.
561 Castano, E. Chacon, C. Chatelain, A. L. Ebel, E. Figueiredo, N. Fuentes, Q. J. Groom, L.
562 Henderson, Inderjit, A. Kupriyanov, S. Masciadri, J. Meerman, O. Morozova, D. Moser, D. L.
563 Nickrent, A. Patzelt, P. B. Pelsler, M. P. Baptiste, M. Poopath, M. Schulze, H. Seebens, W.-s.
564 Shu, J. Thomas, M. Velayos, J. J. Wieringa, and P. Pysek. 2015. Global exchange and
565 accumulation of non-native plants. *Nature* **525**:100-103.
- 566 Van Nuland, M. E., J. K. Bailey, and J. A. Schweitzer. 2017. Divergent plant–soil feedbacks could alter
567 future elevation ranges and ecosystem dynamics. *Nature Ecology & Evolution* **1**:0150.
- 568 Verhoeven, K. J. F., A. Biere, J. A. Harvey, and W. H. van der Putten. 2009. Plant invaders and their
569 novel natural enemies: who is naive? *Ecology Letters* **12**:107-117.
- 570 Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-
571 Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature*
572 **416**:389-395.
- 573 Wang, M., A. Biere, W. H. van der Putten, T. M. Bezemer, and E. P. Brinkman. 2017. Timing of
574 simulated aboveground herbivory influences population dynamics of root-feeding
575 nematodes. *Plant and Soil* **415**:215-228.
- 576 Wilschut, R., S. Geisen, F. Ten Hooven, and W. van der Putten. 2016. Interspecific differences in
577 nematode control between range-expanding plant species and their congeneric natives. *Soil*
578 *Biology and Biochemistry* **100**:233-241.
- 579 Wilschut, R. A., J. C. P. Silva, P. Garbeva, and W. H. van der Putten. 2017. Belowground Plant–
580 Herbivore Interactions Vary among Climate-Driven Range-Expanding Plant Species with
581 Different Degrees of Novel Chemistry. *Frontiers in Plant Science* **8**.
- 582 Yeates, G. W., T. Bongers, R. G. M. Degoede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding-
583 habits in soil nematode families and genera - an outline for soil ecologists. *Journal of*
584 *Nematology* **25**:315-331.

586

587

588 **Figure legends**

589

590 **Fig. 1**

591 Ordination diagrams of principal component analyses (PCA) showing the centroids of
592 nematode community composition based on nematode feeding types (left) and the community
593 of root-feeding nematodes (right). Centroids represent nematode communities in mesocosms
594 inoculated with soils from the original range (filled signs) or new range (open signs), grown
595 with either natives (NAT; squares), related range-expanders (RRE; triangles) or unrelated
596 range-expanders (URE; circles). Arrows represent the relation between nematode feeding types
597 (a) or root-feeding nematode taxa (b) and the variation in nematode community along the PCA-
598 axes. Horizontal and vertical error bars represent standard errors along the first and second
599 PCA-axes. Percentages of total explained variation by the PCA-axes are given in the
600 parentheses.

601 **Fig. 2**

602 Densities of root-feeding (a), bacterivorous (b), omnivorous (c) and fungivorous (d) nematodes
603 in soil (N/100 g dry soil) in mesocosms with native plant species (NAT; white), related range-
604 expanders (RRE; light grey) and unrelated range-expanders (URE; dark grey) in soils from the
605 original range (south) and the new range (north) of the range-expanders. Bars represent
606 averages \pm standard errors. Horizontal bars and asterisks indicate significant differences
607 between soil origins and different letters indicate significant ($p < 0.05$) differences between
608 plant communities within ranges based on Negative binomial GLM and Post-hoc Wald tests.

609 **Fig. 3**

610 Plant community effects on densities in soil (N/100 g dry soil; left) and on roots (N/g root; right)
611 of root-feeding nematode groups *Meloidogyne* (a,e), *Hoplolaimidae* (b,f), *Tylenchidae* (c,g) and

612 *Paratylenchus* (d,h) in soils from the original range and new range. Different bars represent the
613 communities of native plants (NAT; white), related range-expanders (RRE; light grey) and
614 unrelated range-expanders (URE; dark grey). Bars represent averages \pm standard errors.
615 Horizontal bars and asterisks represent significant ($p < 0.05$) differences between soil origins
616 and different letters indicate significant ($p < 0.05$) differences between plant communities
617 within the ranges based on Negative binomial GLM and Post-hoc Wald tests.