

1 **Plant-feeding nematodes in coastal sand dunes: occurrence, host specificity and effects on plant**  
2 **growth**

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14

15 **Abstract**

16

17 *Aims* Coastal sand dunes have a well-established abiotic gradient from beach to land and a  
18 corresponding spatial gradient of plant species representing succession in time. Here, we relate the  
19 distribution of plant-feeding nematodes with dominant plant species in the field to host specialization  
20 and impacts on plant species under controlled greenhouse conditions.

21 *Methods* We assessed plant-feeding nematodes in soil and roots of six plant species that dominate the  
22 vegetation at successional positions along the gradient. In controlled conditions, we determined  
23 performance of all plant-feeding nematodes on each plant species and their effects on plant biomass.

24 *Results* Specialist feeding type nematodes were confined to plant species in either foredunes or  
25 landward dunes. Generalist feeding type nematodes were found in highest numbers in the landward  
26 dunes. Most tested nematode species decreased root, but not shoot or rhizome biomass.

27 *Conclusions* Host plant suitability determined occurrence of some plant-feeding nematodes in dunes,  
28 but abiotic and biotic soil conditions may play a role as well. Generalist feeding type nematodes were  
29 able to reproduce on all plant species. Feeding specialists, which are more protected by plant roots,  
30 might prefer host plants in the foredunes for the same reason as their host plants: to escape from  
31 natural enemies.

32

33 **Key words:** *Ammophila arenaria*, ectoparasite, endoparasite, foredune, generalist, specialist

34

35 **Abbreviation:** Pf/Pi (ratio of final to initial population size)

36

37 **Introduction**

38

39 There is increasing awareness that soil biota play a role as drivers of patterns and spatio-temporal  
40 dynamics in natural vegetation (Bardgett and Wardle 2010). Individual plant species stimulate specific  
41 soil organisms, thereby creating distinct soil communities (Bezemer et al. 2010). Soil biota include an  
42 array of functional types of organisms, including decomposers, mutualistic symbionts, root feeders,  
43 and pathogens. Here, we examine how the distribution of plant-feeding nematodes (also named plant-  
44 parasitic or root-feeding nematodes) in a coastal successional vegetation gradient may relate to host  
45 specificity and to effects on plant biomass production. Plant-feeding nematodes are well known to  
46 cause yield reductions in crop systems (Neher 2010) and vary in degree of host plant specificity (Perry  
47 and Moens 2006; Jones et al. 2013). Coastal sand dunes have been used as a model for plant-soil biota  
48 interactions in general, and plant-nematode interactions in particular (Oremus and Otten 1981;  
49 Seliskar and Huettel 1993; Van der Putten et al. 2006; de la Peña et al. 2008).

50 Coastal sand dunes are characterized by a gradient in environmental conditions from highly  
51 dynamic, salty and poor in organic matter along the beach to more stable, low salt and higher organic  
52 matter landward (De Boer et al. 1998). The gradient in environmental conditions is reflected by a  
53 sequence of different dominant plant species (Huiskes 1979; Doing 1985). The foredunes along the  
54 beach are exposed to deposition of sand by wind and to salt spray from the sea, and plant species that  
55 naturally occur in these sites are adapted to withstand, or even prefer these extreme conditions (Hope-  
56 Simpson and Jefferies 1966; Huiskes 1979; Wilson and Sykes 1999). In North-Western Europe, the  
57 sand dune grass *Ammophila arenaria* is more vigorous in dynamic foredunes that are exposed to  
58 frequent deposition of wind-blown sand from the beach than in stable landward dunes where there is  
59 almost no sand deposition. Sand deposition is thought to provide these plants with an opportunity to  
60 temporarily escape from soil-borne pathogens and plant-feeding nematodes (Van der Putten et al.  
61 1988). When sand burial ceases and *A. arenaria* dies off, other plant species that are insensitive to  
62 soil-borne plant pathogens that affect *A. arenaria* become dominant. Thus, soil-borne plant pathogens  
63 are supposed to promote succession in coastal sand dunes because of being host specific (Van der  
64 Putten et al. 1993).

65 In spite of the studies on occurrence of soil-borne pathogens and plant-feeding nematodes in  
66 coastal sand dunes (De Rooij-van der Goes et al. 1995; Wall et al. 2002), host specificity has not  
67 received much attention yet. In part, it has been difficult to establish which species are potential  
68 pathogens (De Rooij-van der Goes 1995) and how they may be quantified. In the present study, we  
69 focus on plant-feeding nematodes, because they can be relatively easily quantified and cultured. Plant-

70 feeding nematodes have been studied in relation to the ecology of both the European dune grass *A.*  
71 *arenaria* and the North-American congener *Ammophila breviligulata*. In both cases it has been argued  
72 that these nematodes were not the only control agents for the grasses, but that they may operate in  
73 combination with other organisms, such as fungal pathogens (De Rooij-van der Goes 1995; Little and  
74 Maun 1997).

75 Thus far most nematode species tested were either too low in abundance in the field to cause  
76 growth reduction (De Rooij-van der Goes 1995), or they appeared to be bottom-up controlled by the  
77 plant to a non-damaging level (Van der Stoel et al. 2006). However, in some cases nematodes can  
78 have substantial growth reducing effects on their host plant species. For example, in a large-scale field  
79 experiment evidence was provided that the root-knot nematode *Meloidogyne maritima* was able to  
80 reduce biomass production of the dune grass *A. arenaria* when it was added alone. When added in  
81 combination with other plant-feeding nematodes, *M. maritima* did not reduce growth of *A. arenaria*,  
82 probably because it was controlled by competition (Brinkman et al. 2005).

83 Several other studies on factors that may control plant-feeding nematodes in coastal dunes have  
84 pointed at multi-factor controls, including microbes, arbuscular mycorrhizal fungi, and predators  
85 influencing the abundance of plant-feeding nematodes in the root zone of *A. arenaria* (de la Peña et al.  
86 2006b; Hol et al. 2007; Piśkiewicz et al. 2008). However, except for work on the foredune cyst  
87 nematode *Heterodera arenaria* (Van der Stoel and van der Putten 2006) there has been relatively little  
88 attention for the role of host plant specificity as a factor that may determine the presence and  
89 abundance of plant-feeding nematodes in coastal dunes. We combined field surveys with inoculation  
90 studies under controlled conditions in order to single out the role of host plant specificity, or  
91 suitability.

92 Our study had three aims. First, we investigated the occurrence of plant-feeding nematodes in soil  
93 and roots of six plant species that dominate the vegetation gradient from beach to land across coastal  
94 dunes in the Netherlands. Second, we assessed the level of specialization of the nematodes to these  
95 plants under controlled conditions. Specialist nematodes are often endoparasites that enter plants roots  
96 and either feed on inner cell layers or create special feeding sites. Generalists are often ecto- or semi-  
97 endoparasites that feed on the outer cell layers of the root (Perry and Moens 2006; Jones et al.  
98 2013). Third, we determined the effects of the different nematode species on plant biomass production  
99 under controlled conditions. In general, endoparasitic nematodes are more associated with crop losses  
100 than are ectoparasites (Jones et al. 2013). These three aims enabled us to relate nematode distribution  
101 in the root zone of the dominant plant species across the vegetation gradient to their host specialization  
102 and their impacts on the plant species.

103 We tested the hypothesis that nematodes typical of the most dynamic parts of the dunes were the  
104 most specialized to their host plants. The assumption underlying this hypothesis is that extreme abiotic  
105 conditions may not only drive specialization of plants, but also of the plant-feeding nematodes. We  
106 also tested the hypothesis that nematodes from landward dunes would be able to reproduce on a wider  
107 range of plant species in the vegetation gradient. The underlying assumption for this hypothesis is that  
108 generalist nematodes may not develop populations on the early successional plant species because of  
109 the extreme conditions near the beach. The data used for the present study originate from a series of  
110 separate experiments. A small fraction of the data are already published (Van der Stoel and van der  
111 Putten 2006), but are also included in the present work (see methods) in order to present a  
112 comprehensive study on linking occurrence, specificity, and effectiveness of plant-feeding nematodes  
113 in a natural ecosystem.

## 115 **Materials and methods**

### 117 **Field survey**

119 Soil samples were taken from the root layer of six monocotyledonous plant species that grow on  
120 coastal dunes at Voorne, the Netherlands. The plant species that we sampled are, in successional order  
121 from the beach towards the inner dunes: *Elytrigia juncea* (L.) Nevski subsp. *boreoatlantica* (Simonet  
122 & Guin.) N.Hyl. (sand couch), *Ammophila arenaria* (L.) Link (marram grass; vigorous and  
123 degenerated stage), *Festuca arenaria* Osbeck (sand fescue), *Carex arenaria* L. (sand sedge), *Elytrigia*  
124 *atherica* (Link) Kerguelen (sea couch) and *Calamagrostis epigejos* (L.) Roth (wood small-reed). All

125 sampled plant species occur naturally along the coast of North-Western Europe. They are perennial,  
126 grow in relatively large mono-specific stands, and they all have an optimum abundance in a specific  
127 vegetation zone (Doing 1985; Van der Laan et al. 1997). Sampling in a long-lived monospecific stand  
128 was supposed to provide a good indication of which nematodes could be supported by that particular  
129 plant species. The samples were taken along four approximately 150 m long transects from the  
130 foredune to the inner dunes at each of two sites: Oostvoorne ‘car beach’ (dune with approximately 5  
131 cm annual sand accretion; 51°9’ N 04°06’ E) and ‘Haringvlietdam’ (dune with approximately 20 cm  
132 annual sand accretion; 51°52’ N 04°04’ E). To ascertain detection of all nematode species, including  
133 those that may have low densities at certain times of the year (Van der Stoel et al. 2002), soil samples  
134 were collected on four dates (April, June, August and October 1996). Each replicate consisted of two  
135 combined samples of about 1.5 L soil collected within 2m distance from a depth of 0.10-0.15 m  
136 underneath the soil surface. When the newly deposited upper soil layer did not yet contain roots, this  
137 layer was removed until the first roots appeared. The soil sample was then collected from the rooted  
138 sublayer and stored at 4 °C until processing.

139 Roots were separated from the soil by sieving through 1.0 and 0.5 cm mesh sizes, after which the  
140 soil was gently homogenized. Nematodes were extracted from a sample of 0.25 L soil (density ca. 1.4  
141 kg/L soil) by Oostenbrink elutriation (Oostenbrink 1960), followed by decantation onto a stack of one  
142 75 µm and three 45 µm sieves. To collect free-living nematodes, the debris on the sieves was  
143 transferred to a double cotton milk filter (Hygia rapid, Hartmann AG, Heidenheim, Germany) held by  
144 a tray in a dish with a layer of tap water. The nematodes were allowed to pass through the filter for 24  
145 h and were then stored at 4 °C until counting. When roots of a different plant species were present in  
146 the sample, these were removed prior to extraction. Nematodes were extracted from the roots by the  
147 funnel spray method for 48 h (Oostenbrink 1960). For identification and counting, 5 out of 100 ml  
148 suspension was used from the soil extracts and 5 out of 50 ml from the root extracts. Only plant-  
149 feeding nematodes, which were the focus of the study, were counted and identified to genus or species  
150 level according to Bongers (1988).

151

#### 152 Inoculation experiments

153

154 All the plant species that were sampled in the field survey were tested for host suitability. Seeds of the  
155 different plant species were collected at the ‘Haringvlietdam’ field site (see Field survey). The seeds  
156 were germinated on moist glass beads at 25/15 °C at a 16/8 h light/dark regime. The seedlings were  
157 planted in 1.5 L pots filled with 1500 g sterilized (25 kGray) beach sand with a moisture content of 10  
158 % (w w<sup>-1</sup>). They were grown in a greenhouse with daytime temperature of 21 ± 2 °C and night  
159 temperature of 19 ± 2 °C. The plants were provided with extra light to ensure a minimum  
160 photosynthetic photon fluence rate of 200 µmol m<sup>-2</sup> s<sup>-1</sup> over the waveband 400-700 nm at daytime.  
161 Four seedlings were planted per pot, except when adding *Meloidogyne duiysi*, *Meloidogyne maritima*  
162 and *Pratylenchus* spp. (a mixture of *P. dunensis* and *P. brzeskii*) three seedlings were planted per pot  
163 (for no specific reason, except that we planted more than one seedling per pot in order to produce more  
164 biomass).

165 Two weeks after planting, nematodes were inoculated close to the roots of each plant in the pot.  
166 Nematodes for the inoculation were extracted from cultures that were maintained on *A. arenaria* in the  
167 greenhouse, except for *Heterodera arenaria*, *M. duiysi* and *M. maritima*, of which egg-bearing  
168 females were collected from the field. The number of nematodes that was inoculated to each pot varied  
169 due to limited availability of some species: 200 individuals (*Helicotylenchus pseudorobustus*,  
170 *Hemicycliophora conida*, *Mesocriconema xenoplax*, *Rotylenchus goodeyi*, *Neodolichorhynchus*  
171 *microphasmis* and *Telotylenchus ventralis* (when added to *E. juncea*)); 400 (*M. duiysi*, *M. maritima*  
172 and *Pratylenchus* spp.); 476 (*T. ventralis* when added to the other plant species; inoculum was added  
173 twice with one week intercept due to low recovery); 1700 (juveniles of *H. arenaria*). Tap water was  
174 added in the same manner to the control plants. The inoculations with the different nematode species  
175 were not all performed at the same time. However, in every experiment with a particular nematode  
176 species all plant species were inoculated with that nematode species and a control without nematode  
177 addition was included. Five replicates per treatment were used, except for the test with *H. arenaria*  
178 when six replicates were used. The results of the test with *H. arenaria* have been published separately  
179 (expressed in numbers per amount of root biomass; Van der Stoel and van der Putten 2006), but we

180 expressed the numbers per amount of soil and added them to the current overview for completeness.  
181 When relevant, we have indicated the source of the published data on *H. arenaria* in the Tables. Two  
182 times per week, the soil moisture content was re-set with demineralized water to 10 % (w w<sup>-1</sup>) by  
183 weighing. Hoagland nutrient solution was added weekly in an increasing amount to meet the demands  
184 of the growing plant (weeks 1 to 5: 25 ml half strength; weeks 5 and 6: 25 ml full strength (1H); weeks  
185 7 and 8: 50 ml 1H and from 9 weeks onwards: 75 ml 1H; but for the experiment with *H. arenaria*  
186 weeks 1 to 6: 12.5 ml 1H, weeks 7 to 11: 25 ml 1H and weeks 12 and 13: 50 ml 1 H per pot).

187 The plants were harvested 10-14 weeks after nematode inoculation. The sand was washed from  
188 the roots using tap water and shoots were separated from the roots. The plant material was dried at 70  
189 °C for 48 h and then weighed. Nematodes were extracted from the sand by decantation. Tap water was  
190 added to the soil slurry to obtain a volume of 5 L. The suspension was stirred, after which the water  
191 was poured onto a stack of one 75 µm and three 45 µm sieves. This procedure was performed four  
192 times. The extraction of the nematodes from the debris on the sieves was the same as described above  
193 for the field survey. For extraction of cysts (females) of *H. arenaria*, the suspension was poured onto a  
194 180 µm sieve and the debris was collected on filter paper. The contents were air-dried and then  
195 visually inspected for cysts using a stereomicroscope. Due to very poor growth of *C. arenaria* in the  
196 inoculation experiment with *H. arenaria*, that part of the experiment was terminated at an early stage  
197 before female cysts could have developed (data not shown in Tables 2 and 4).

198

#### 199 Data analysis

200

201 For the greenhouse experiments, data were only compared that originated from experiments that were  
202 performed at the same time. Final nematode numbers in the greenhouse experiment were ln-  
203 transformed to achieve homogeneity of variances, after which the results were analyzed by one-way  
204 ANOVA with plant species as factor. Biomass of plants was ln-transformed to achieve homogeneity  
205 of variances. Effects of plant species and nematode addition (yes/no) on plant biomass were analyzed  
206 by two-way ANOVA. When transformation did not result in homogeneous variances, the results were  
207 analyzed by non-parametric Scheirer-Ray-Hare test, which is a two-way extension of the Kruskal-  
208 Wallis test (Sokal and Rohlf 1995). Approximate P-values for the Scheirer-Ray-Hare test were  
209 obtained from Rohlf and Sokal (1981). The correlation between the successional range of plant species  
210 and numbers of co-occurring endoparasites and ecto- and semi-endoparasites, as well as Pf/Pi (=ratio  
211 of final to initial population size in the inoculation experiment) and nematode addition effects on shoot  
212 and root biomass, was tested with Spearman's rank-order correlation using Statistica 10. Pf/Pi of *H.*  
213 *arenaria* was left out from the analysis as only numbers of first generation female cysts were  
214 determined and not the offspring that they contained.

215

## 216 Results

217

### 218 Ecto- and semi-endoparasites

219

220 In the field, ecto- and semi-endoparasitic nematodes were found on all the plant species that we  
221 sampled (Table 1). However, potential multiplication in the inoculation experiment showed different  
222 reproductive capacities than would be predicted from the densities in the field. In the field, *H. conida*  
223 and *H. pseudorobustus* mainly were associated with *C. epigejos* (Table 1). However, in the greenhouse  
224 reproduction of *H. conida* did not differ significantly among the six plant species, whereas  
225 reproduction of *H. pseudorobustus* was only intermediate on *C. epigejos* (Tables 2 and 3).  
226 Criconeematidae (*Criconemoides amorphus* and *M. xenoplax*; the two species were not distinguished  
227 during counting) in the field were detected in low densities on all plant species except for *E. juncea*  
228 (Table 1). However, in the greenhouse experiment, *M. xenoplax* reproduced on all plant species,  
229 although less so on *F. arenaria* (Tables 2 and 3). In the field, *R. goodeyi* was detected on all plant  
230 species except for vigorous *A. arenaria*, although densities were higher in the inner dunes (Table 1). In  
231 the greenhouse, numbers remained low on all plant species (Tables 2 and 3). In the field, *N.*  
232 *microphasmis* and/or *Geocenamus nanus* (the two species were not distinguished during counting)  
233 were found on all plant species, although densities were higher in the inner dunes (Table 1). In the  
234 greenhouse experiment, *N. microphamis* reproduced on all plant species, although reproduction was

235 lower on *E. atherica*. In the field, *T. ventralis* was found in low densities on all plant species (Table 1).  
236 In the greenhouse, reproduction was high on all plant species except *C. arenaria* (Tables 2 and 3).

237 The density of ectoparasites and semi-endoparasites associated to the plant species in the dune  
238 succession gradient significantly increased from beach to land (Fig. 1a; Spearman  $R=0.340$ ,  $P <$   
239  $0.001$ ). When inoculated to the six plant species, Pf/Pi of the ecto- and semi-endoparasites was not  
240 significantly related to the successional order of the plant species in the coastal dunes (Fig. 1b;  
241 Spearman  $R=-0.046$ ,  $P=0.54$ ).

242

243 Endoparasites

244

245 The genera *Heterodera*, *Meloidogyne* and *Pratylenchus* were detected on all the plant species included  
246 in our study (Table 1). However, in the field survey that we present here, different nematode species  
247 had not yet been recognized. When tested in the greenhouse experiment, *H. arenaria* mainly  
248 reproduced on *E. juncea*, *A. arenaria* and on *C. epigejos* (Tables 2 and 3). *Meloidogyne duytisi*  
249 reproduced on the congeners *E. juncea* and *E. atherica*, whereas *M. maritima* reproduced only on *A.*  
250 *arenaria*. The *Pratylenchus* spp. that we tested, presumably a mixture of *P. brzeskii* and *P. dunensis*,  
251 produced high numbers on *E. juncea* and *A. arenaria*, whereas numbers remained low on the other  
252 plant species.

253 In contrast to the ectoparasites and semi-endoparasites, the density of endoparasites in the field  
254 was low (as is usual in the coastal dunes) and did not significantly correlate with the succession of  
255 plant species in the dunes (Spearman  $R=0.074$ ,  $P=0.25$ ). However, when added to the same range of  
256 plant species, Pf/Pi of the endoparasites tended to decrease along the successional gradient from the  
257 sea landward (Fig. 1b; Spearman  $R= -0.179$ ,  $P=0.09$ ). This was mainly due to the relatively high  
258 reproduction of *Pratylenchus* spp. on *E. juncea* and *A. arenaria* (Fig. 1b).

259

260 Effects on plants

261

262 In the greenhouse experiment, all nematode species except *Pratylenchus* spp. significantly decreased  
263 root biomass of the tested plant species. However, pairwise comparisons between control plants and  
264 inoculated plants in most cases were not significantly different, except for *T. ventralis* and, to a lesser  
265 extent, *H. arenaria* (Tables 4 and 5). Most nematode species that we added to the plant species did not  
266 have a significant effect on shoot and rhizome biomass. Only *T. ventralis* significantly decreased shoot  
267 biomass of *A. arenaria* and *C. epigejos*, and rhizome biomass of *E. atherica* and *C. epigejos* (Tables 4  
268 and 5). Considering the effects of the nematodes on plant biomass production, the ecto- and semi-  
269 endoparasitic plant-feeding nematodes had a more negative effect on shoot biomass of plant species  
270 from the landward part of the succession gradient than on plant species from foredunes (Spearman  $R=$   
271  $-0.228$ ,  $P=0.002$ ). In contrast, the effects of the endoparasites on shoot biomass did not show a  
272 significant trend (Spearman  $R=0.111$ ,  $P=0.23$ ). Neither endoparasites (Spearman  $R=0.144$ ,  $P=0.28$ ),  
273 nor ecto- and semi-endoparasites (Spearman  $R= -0.022$ ,  $P=0.77$ ) showed a relationship between  
274 position of the plant species in the successional gradient and effect on root biomass.

275

276 **Discussion**

277

278 In line with our hypothesis, we found that specialist plant-feeding nematodes were mostly confined to  
279 plant species that occur in the dynamic foredunes along the beach. On the other hand, generalist plant-  
280 feeding nematodes were detected with plants throughout the whole dune gradient, but densities were  
281 lower in the dynamic foredunes than in the stabilized dunes. During our survey, several of the plant-  
282 feeding nematode species turned out to be undescribed (Karssen et al. 1998b; Karssen et al. 2000; de  
283 la Peña et al. 2006a). The inoculation experiments confirmed the high level of specialization of plant-  
284 feeding nematodes that thus far have only been found in coastal dunes. Here, we discuss our results in  
285 relation to current knowledge on occurrence and specialization of plant-feeding nematodes in coastal  
286 dunes. We discuss the various mechanisms other than host specialization, for example biotic  
287 interactions and abiotic environmental factors, that may influence the observed distribution of plant-  
288 feeding nematodes in the dunes.

289 In our survey, several endoparasitic nematode genera were detected in the root zone of all the  
290 plant species that we sampled. The level of specificity was highest for nematodes that occurred with  
291 plants in the dynamic foredunes, especially *E. juncea* and *A. arenaria*. Cyst nematodes (*Heterodera*  
292 spp.) were detected with plants throughout the entire dune gradient, but were likely represented by  
293 different species. The sedentary endoparasite *H. arenaria* appeared to be confined to plants in the  
294 foredunes that are exposed to sand deposition and salt spray. Landward, *H. arenaria* became gradually  
295 replaced by *H. hordecalis* (Fig. 2; Clapp et al. 2000; Van der Stoel and van der Putten 2006). Also root  
296 knot nematodes (*Meloidogyne* spp.) were detected in the root zone of all studied plant species, but  
297 probably different plant species hosted different root knot nematode species. For example, *M. duytsi*  
298 was found predominantly, if not exclusively on *E. juncea*, *M. maritima* on *A. arenaria*, whereas non-  
299 identified *Meloidogyne* spp. other than *M. duytsi* and *M. maritima* were isolated from plant species  
300 with a more landward occurrence. The migratory endoparasites *Pratylenchus* spp. were found  
301 throughout the dune succession gradient as well, although it is more difficult to link their species  
302 names to host plant species. The migratory endoparasites *P. brzeskii* and *P. dunensis* have been  
303 described to occur on *E. juncea* and *A. arenaria*, whereas *P. brzeskii* also has been detected on *Leymus*  
304 *arenarius* (L.) Hochst. (Karssen et al. 2000; de la Peña et al. 2006a). From our own observations,  
305 *Pratylenchus* spp. also occur on the other plant species, however, their species names have not yet  
306 been established.

307 The host range of the endoparasitic nematode species ascertained from the field survey did not  
308 fully correspond to the host range as determined by the inoculation experiment. In the greenhouse, the  
309 level of host plant specificity increased in the following order: *H. arenaria*, *P. brzeskii* and/or *P.*  
310 *dunensis*, *M. duytsi* and *M. maritima*. We found that *H. arenaria* was capable to reproduce on both *E.*  
311 *juncea* and on *A. arenaria*, but also on *C. epigejos*, whereas in the field this nematode species only  
312 occurred on plant species in the foredunes (Van der Stoel and van der Putten 2006). Thus, host  
313 suitability is not the only factor that determines the occurrence of *H. arenaria* in the foredunes. It  
314 might be that *H. arenaria* prefers dynamic abiotic conditions, such as sand deposition and salt spray,  
315 but these abiotic factors have not been included in the greenhouse experiments. Alternatively, like the  
316 host plant *A. arenaria*, the dynamic foredune environment might enable the cyst nematode *H. arenaria*  
317 to escape from natural enemies (Piśkiewicz et al. 2008). The specificity of the migratory endoparasites  
318 *P. dunensis* and *P. brzeskii* to *E. juncea* and *A. arenaria* suggests that they are restricted to foredunes  
319 due to the ecology of their host plants (de la Peña et al. 2008).

320 To our knowledge, dune vegetation is the only natural habitat for both *M. duytsi* and *M. maritima*,  
321 and the host plant preference of these two root knot species is rather specific compared to many other  
322 species within this nematode genus (Jones et al. 2013). As *M. duytsi* in our inoculation experiment did  
323 not reproduce on *A. arenaria*, it is likely that the reported occurrence on *A. arenaria* in Karssen et al.  
324 (1998b) originated from intermingled growth of the roots of *A. arenaria* and *E. juncea*. Interestingly, in  
325 the field *M. duytsi* has been found on *E. juncea*, but in the greenhouse it was able to reproduce as well  
326 on the congener *E. athericus*. This suggests that the occurrence of *M. duytsi* in foredunes is due to  
327 preference of dynamic abiotic conditions, or escape from natural enemies under those conditions. The  
328 results of our inoculation experiment suggest that *A. arenaria* is the only host plant of *M. maritima*,  
329 although the distinction between hosts and non-hosts may be obscured by overall low numbers of  
330 juveniles and males at the end of the experiment. Previously, *M. maritima* had been detected on *A.*  
331 *arenaria*, *L. arenarius* and *C. epigejos* (Karssen et al. 1998a). However, it is likely that *M. maritima*  
332 was feeding only on roots of *A. arenaria* that can still be present as remnant plants that grow  
333 intermingled with the other plant species in landward dune sites.

334 The ectoparasitic and semi-endoparasitic nematodes in our tests were generalists: they were  
335 capable of reproducing on the full range of coastal dune plant species, although most of them  
336 reproduced less well on one of the plant species. Therefore, the prevalence of ectoparasites in the  
337 landward dunes is likely caused by other factors than the availability of specific host plants. The host  
338 range of *H. pseudorobustus* is wide and it has a cosmopolitan distribution (Verschoor et al. 2001a;  
339 Davis et al. 2004; Silva et al. 2008). The species is dominant in high-productive grasslands, but  
340 decreases after cessation of fertilization (Verschoor et al. 2001b). That may be an important reason  
341 why *H. pseudorobustus* was found in highest numbers in landward dunes, where nutrient availability  
342 may be higher than close to the beach. *Rotylenchus goodeyi* is usually found in low numbers in many  
343 different vegetation types and it seems to have a long generation time (Boag and Neilson 1996), as

344 also was indicated by the low reproduction in our greenhouse specificity trial. The slow reproduction  
345 may be disadvantageous in the dynamic dunes close to the beach, where ample offspring might be  
346 necessary insurance against extreme abiotic conditions. *Hemicycliophora conida*, *M. xenoplax* and *N.*  
347 *microphasmis* all have been found on woody plant species (Loof 1984; Zoon et al. 1993; Nico et al.  
348 2002) and may not be adapted to the dynamics of the foredunes. *Telotylenchus ventralis* was first  
349 described from the roots of rye, oats and rye-grass (Loof 1963) and thus is not specific for coastal  
350 dunes. In the field survey, densities of *T. ventralis* were low on all studied plant species, whereas in  
351 the greenhouse reproduction was high on the grasses, but low on *C. arenaria*. The species may not  
352 thrive well in the abiotic conditions of sand dunes. Further experiments are needed to verify how  
353 abiotic conditions like salinity, nutrient and organic matter content and sand accretion may affect the  
354 occurrence of the nematodes (Nkem et al. 2006; Erb and Lu 2013).

355 The occurrence of plant-feeding nematode species may not only depend on host plant suitability  
356 and abiotic conditions (Mateille et al. 2011), but also on interactions with other organisms (Erb and Lu  
357 2013). Many nematode species that occur in the dunes can be suppressed by micro-organisms present  
358 in the root zone of *A. arenaria* (de la Peña et al. 2006b; Piśkiewicz et al. 2008; Costa et al. 2012). It is  
359 not known if micro-organisms from the root zone of other plant species are suppressing *T. ventralis* to  
360 the same extent. Nematodes may also be suppressed by competition with co-occurring species: for  
361 example, in a previous study reproduction of *M. maritima* was delayed and reduced when *H. arenaria*  
362 and *P. penetrans* were added to the same plants (Brinkman et al. 2005). However, the opposite  
363 occurred when addition of *T. microphasmis* facilitated reproduction of *T. ventralis* on the non-host  
364 plant *C. arenaria* (Brinkman et al. 2008). Therefore, in order to further understand why some  
365 nematodes do not occur with potentially suitable host plant species, or why they occur at relatively  
366 low numbers in the field, additional studies are needed on biotic controls of these nematodes in the  
367 various successional stages of dune soil development.

368 For the nematodes to play a role in succession, they either need to be specific to certain plant  
369 species, or vary in effect strengths. In the inoculation experiment, most nematode species decreased  
370 root biomass, but did not affect shoot and rhizome biomass of the plant species. However, within-plant  
371 species comparisons of inoculated and control plants mostly were not significant. Exceptions were *T.*  
372 *ventralis* and *H. arenaria* that decreased shoot, rhizome or root biomass of several plant species,  
373 conform effects reported in previous studies (De Rooij-van der Goes 1995; Brinkman et al. 2004). In  
374 general, ecto- and semi-endoparasites decreased shoot biomass of plant species from landward dunes  
375 more than from foredunes. This may appear contrary to the hypothesis that plant species from  
376 foredunes benefit from sand burial because they need to escape from natural enemies in lower soil  
377 layers. However, in the field plants are attacked by a mixture of plant-feeding nematode and  
378 pathogenic microorganisms that co-occur and possibly interact with each other (De Rooij-van der  
379 Goes 1995; Brinkman et al. 2005, 2008). Hitherto unknown interactions with other suppressive soil  
380 organisms may further limit the extrapolation of our results to field conditions (Van der Stoel and van  
381 der Putten 2006; Piśkiewicz et al. 2008).

382 In conclusion, our data are largely in support of our two hypotheses: specialist plant-feeding  
383 nematodes were confined to plant species in the dynamic foredunes, but they may also reproduce on  
384 plant species from the same genus that occur in landward dunes. Generalist plant-feeding nematodes  
385 indeed were found in higher numbers in the less extreme -landward- environment, but under  
386 controlled conditions they were able to reproduce on all plant species. Therefore, it appears that in  
387 coastal dunes specialized nematodes are largely controlled by host plants, whereas generalist  
388 nematodes are controlled by extreme abiotic factors. In addition, our study and that of others (e.g. De  
389 Rooij-van der Goes 1995; de la Peña et al. 2006b; Piśkiewicz et al. 2008) strongly suggests that other  
390 (abiotic, but also biotic) factors may play an additional role in nematode control in coastal sand dune  
391 soil.

## 392 **Acknowledgements**

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396  
397



Table 1. Occurrence (mean number per 100 g dry soil  $\pm$  s.e ) of plant-feeding nematodes in the root zone of different plant species at dune locations with little (Oostvoorne 'car beach') or more (Haringvlietdam) annual sand accretion in the The Netherlands (n=16; averaged over four sample points and four sample dates).

<u>Location/feeding group</u> Nematode	<i>E. juncea</i>	<i>A. arenaria</i> (vigorous)	<i>A. arenaria</i> (degenerating)	<i>F. arenaria</i>	<i>C. arenaria</i>	<i>E. atherica</i>	<i>C. epigejos</i>
<u>Oostvoorne 'car beach'</u>							
Ectoparasites and semi-endoparasites							
Criconematidae	0	0	1.4 $\pm$ 0.64	3.6 $\pm$ 2.55	3.2 $\pm$ 1.80	2.9 $\pm$ 1.58	6.9 $\pm$ 3.72
<i>Helicotylenchus pseudorobustus</i>	0	0	0.4 $\pm$ 0.36	9.0 $\pm$ 7.00	0	0.0 $\pm$ 0.04	24.3 $\pm$ 22.80
<i>Hemicycliophora conida</i>	0	0.3 $\pm$ 0.30	1.8 $\pm$ 1.02	0	2.5 $\pm$ 2.15	10.4 $\pm$ 5.66	41.4 $\pm$ 13.78
<i>Rotylenchus goodeyi</i>	0.4 $\pm$ 0.36	0	0	3.6 $\pm$ 1.98	0	1.3 $\pm$ 0.83	0.1 $\pm$ 0.14
<i>Neodolichorhynchus microphasmis/</i> <i>Geocenamus nanus</i>	1.4 $\pm$ 0.82	4.8 $\pm$ 2.67	2.9 $\pm$ 1.56	43.1 $\pm$ 10.39	25.5 $\pm$ 6.69	28.3 $\pm$ 5.89	19.0 $\pm$ 6.35
<i>Telotylenchus ventralis</i>	0.0 $\pm$ 0.02	1.0 $\pm$ 0.60	0.7 $\pm$ 0.49	0.1 $\pm$ 0.07	0.7 $\pm$ 0.49	0.1 $\pm$ 0.09	0.1 $\pm$ 0.14
Endoparasites							
<i>Heterodera</i> spp. (juv.)	0.4 $\pm$ 0.40	7.5 $\pm$ 4.02	2.5 $\pm$ 1.38	0	0	0.8 $\pm$ 0.71	6.6 $\pm$ 3.32
<i>Meloidogyne</i> spp. (juv.)	1.8 $\pm$ 0.77	3.5 $\pm$ 1.58	2.9 $\pm$ 1.56	21.9 $\pm$ 7.28	0.7 $\pm$ 0.71	1.1 $\pm$ 0.78	7.6 $\pm$ 2.65
<i>Pratylenchus</i> spp.	0.2 $\pm$ 0.19	10.1 $\pm$ 3.80	0.7 $\pm$ 0.68	7.6 $\pm$ 2.19	0.4 $\pm$ 0.20	2.3 $\pm$ 1.06	11.5 $\pm$ 2.55
<u>Haringvlietdam</u>							
Ectoparasites and semi-endoparasites							
Criconematidae	0	0.3 $\pm$ 0.29	0.0 $\pm$ 0.02	0	0	0.4 $\pm$ 0.36	0.6 $\pm$ 0.56
<i>Helicotylenchus pseudorobustus</i>	0.4 $\pm$ 0.36	0.3 $\pm$ 0.29	0	0	0.8 $\pm$ 0.76	0.8 $\pm$ 0.46	17.2 $\pm$ 13.96
<i>Hemicycliophora conida</i>	0.0 $\pm$ 0.04	0	0	0	0	0.0 $\pm$ 0.05	1.2 $\pm$ 0.91
<i>Rotylenchus goodeyi</i>	0.8 $\pm$ 0.71	0	0.4 $\pm$ 0.36	0	3.7 $\pm$ 2.93	0.8 $\pm$ 0.49	19.5 $\pm$ 12.93
<i>Neodolichorhynchus</i>	3.2 $\pm$ 0.89	1.6 $\pm$ 0.65	2.9 $\pm$ 1.81	1.1 $\pm$ 0.58	2.6 $\pm$ 1.47	1.8 $\pm$ 1.45	4.7 $\pm$ 2.33

<i>microphasmis/</i>							
<i>Geocenamus nanus</i>							
<i>Telotylenchus ventralis</i>	0.8 ± 0.47	0.8 ± 0.42	0.9 ± 0.50	0.4 ± 0.36	0	0	0.6 ± 0.57
Endoparasites							
<i>Heterodera</i> spp. (juv.)	1.2 ± 1.07	8.8 ± 3.29	1.4 ± 0.94	0.4 ± 0.36	0.7 ± 0.49	0.4 ± 0.36	0.6 ± 0.39
<i>Meloidogyne</i> spp. (juv.)	2.8 ± 1.47	3.4 ± 0.97	6.6 ± 2.36	0	0	1.8 ± 1.79	1.2 ± 0.95
<i>Pratylenchus</i> spp.	4.3 ± 1.50	0.5 ± 0.30	0.5 ± 0.36	0.0 ± 0.03	0.1 ± 0.07	15.4 ± 5.37	6.4 ± 2.25

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Table 2. Final nematode numbers per pot when added to different plant species (n=5; *H. arenaria* n=6). Different letters indicate significant differences in nematode numbers within a row. Numbers of *H. arenaria* have been recalculated from Van der Stoel and van der Putten (2006).

Nematode	Plant species					
	<i>E. juncea</i>	<i>A. arenaria</i>	<i>F. arenaria</i>	<i>C. arenaria</i>	<i>E. atherica</i>	<i>C. epigejos</i>
Ectoparasites and semi-endoparasites						
<i>H. pseudorobustus</i>	630 <sup>ab</sup>	728 <sup>b</sup>	734 <sup>b</sup>	1042 <sup>b</sup>	398 <sup>a</sup>	606 <sup>ab</sup>
<i>H. conida</i>	340 <sup>a</sup>	1458 <sup>a</sup>	315 <sup>a</sup>	642 <sup>a</sup>	788 <sup>a</sup>	1270 <sup>a</sup>
<i>M. xenoplax</i>	5672 <sup>ab</sup>	6546 <sup>b</sup>	1314 <sup>a</sup>	3582 <sup>ab</sup>	4354 <sup>ab</sup>	5830 <sup>ab</sup>
<i>R. goodeyi</i>	318 <sup>a</sup>	234 <sup>a</sup>	180 <sup>a</sup>	130 <sup>a</sup>	154 <sup>a</sup>	356 <sup>a</sup>
<i>N. microphasmis</i> <sup>x</sup>	(5844)	3300 <sup>b</sup>	3723 <sup>b</sup>	5459 <sup>b</sup>	1639 <sup>a</sup>	4348 <sup>b</sup>
<i>T. ventralis</i> <sup>x</sup>	(34600)	8859 <sup>ab</sup>	14864 <sup>b</sup>	748 <sup>a</sup>	6817 <sup>ab</sup>	15040 <sup>b</sup>
Endoparasites						
<i>H. arenaria</i> <sup>y</sup>	33.5 <sup>b</sup>	37.0 <sup>b</sup>	1.7 <sup>a</sup>	n.d.	2.3 <sup>a</sup>	12.8 <sup>ab</sup>
<i>M. duytsi</i> <sup>z</sup>	599 <sup>ab</sup>	0 <sup>a</sup>	1.2 <sup>ab</sup>	0.6 <sup>ab</sup>	1274 <sup>b</sup>	0.4 <sup>ab</sup>
<i>M. maritima</i> <sup>z</sup>	0.2 <sup>a</sup>	25 <sup>b</sup>	1.4 <sup>a</sup>	0.8 <sup>a</sup>	0.6 <sup>a</sup>	0 <sup>a</sup>
<i>Pratylenchus spp.</i>	1.64 · 10 <sup>5</sup> <sup>c</sup>	8.98 · 10 <sup>4</sup> <sup>c</sup>	137 <sup>b</sup>	32 <sup>a</sup>	102 <sup>b</sup>	405 <sup>b</sup>

<sup>x</sup> The experiment with addition of *N. microphasmis* and *T. ventralis* to *E. juncea* was performed at a different moment than addition of these two nematodes to the other plants and was therefore excluded from the statistical analysis; <sup>y</sup> Female cysts; <sup>z</sup> Juveniles and males. N.d.: not determined.

Table 3. Results of one-way ANOVA (with F-value) or Kruskal-Wallis (with H-value) testing the effect of plant species on final nematode numbers. Significant effects are presented in bold.

Nematode	F- or H-value	P-value	MS <sub>error</sub>
Ectoparasites and semi-endoparasites			
<i>H. pseudorobustus</i>	F <sub>5,24</sub> =6.900	<b>0.0004</b>	0.090
<i>H. conida</i>	F <sub>5,23</sub> =2.692	<b>0.0467</b>	0.726
<i>M. xenoplax</i>	H <sub>5, N=30</sub> =12.45	<b>0.0292</b>	-
<i>R. goodeyi</i>	F <sub>5,24</sub> =2.524	0.0569	0.340
<i>N. microphasmis</i>	F <sub>4,20</sub> =11.71	<b>0.0000</b>	0.090
<i>T. ventralis</i>	H <sub>4, N= 25</sub> =15.97	<b>0.0031</b>	-
Endoparasites			
<i>H. arenaria</i> (cysts)	H <sub>4, N= 28</sub> =21.03	<b>0.0003</b>	-
<i>M. duytsi</i>	H <sub>5, N=30</sub> =19.60	<b>0.0015</b>	-
<i>M. maritima</i>	F <sub>5,24</sub> =6.920	<b>0.0004</b>	0.702
<i>Pratylenchus spp.</i>	F <sub>5,24</sub> =75.01	<b>0.0000</b>	1.067

Table 4. Effect of addition of different nematode species on shoot, rhizome and root biomass ( $\text{g} \cdot \text{pot}^{-1}$ ) of six plant species. Values are backtransformed means. As we compiled data from several experiments, biomass of 5 control treatments (Control 1...5) is presented above the inoculation treatments that were performed at the same time. Significant main effects of nematode addition (i.e. overall effects on the whole range of plant species) are presented as nematode species names in bold, and significant contrasts between inoculated and control plants are presented as plant biomasses in bold ( $n=5$ , except *H. arenaria*  $n=6$ ). Biomass of *H. arenaria* has been published in Van der Stoel and van der Putten (2006).

Plant part Nematode	Plant species					
	<i>E. juncea</i>	<i>A. arenaria</i>	<i>F. arenaria</i>	<i>C. arenaria</i>	<i>E. athericus</i>	<i>C. epigejos</i>
<b>Shoot</b>						
Control 1	5.45 (4.72-6.28)	7.51 (7.06-8.00)	5.32 (4.82-5.87)	3.92 (3.70-4.16)	3.86 (3.13-4.77)	5.44 (4.07-7.28)
<i>H. pseudorobustus</i>	5.54 (4.69-6.53)	7.00 (6.49-7.55)	5.08 (4.60-5.62)	3.87 (3.47-4.32)	3.91 (3.44-4.43)	4.98 (4.41-5.62)
<i>H. conida</i>	5.65 (5.20-6.15)	6.98 (6.48-7.51)	5.15 (4.41-6.02)	3.94 (3.69-4.21)	3.53 (2.85-4.37)	4.80 (4.17-5.53)
<i>M. xenoplax</i>	6.32 (5.48-7.30)	7.28 (6.51-8.14)	5.39 (4.82-6.03)	3.86 (3.73-3.99)	3.56 (3.13-4.05)	5.06 (4.61-5.55)
<i>R. goodeyi</i>	<b>6.78 (5.79-7.94)</b>	7.01 (6.50-7.57)	5.46 (4.74-6.29)	3.80 (3.66-3.95)	3.78 (3.28-4.35)	4.92 (4.58-5.28)
Control 2		3.34 (2.92-3.81)	2.68 (2.40-2.99)	2.27 (2.17-2.38)	2.79 (2.54-3.07)	2.50 (2.33-2.68)
<i>N. microphasmis</i> <sup>a</sup>	5.41 (4.29-6.82)	3.11 (2.62-3.68)	2.61 (2.39-2.85)	2.18 (1.78-2.68)	2.48 (2.34-2.62)	2.52 (2.26-2.80)
Control 3		1.69 (1.51-1.88)	1.50 (1.20-1.86)	1.90 (1.67-2.15)	2.51 (2.33-2.70)	1.87 (1.48-2.36)
<i>T. ventralis</i> <sup>a</sup>	5.70 (4.81-6.76)	<b>0.88 (0.53-1.45)</b>	1.23 (0.91-1.65)	1.81 (1.65-1.98)	2.48 (2.06-2.98)	<b>1.15 (0.80-1.66)</b>
Control 4	5.67 (5.31-6.04)	5.89 (4.96-6.99)	6.71 (5.28-8.53)	n.d.	5.21 (4.29-6.33)	3.95 (3.20-4.89)
<i>H. arenaria</i>	5.70 (5.08-6.39)	5.71 (5.27-6.19)	6.60 (5.88-7.40)	n.d.	4.49 (3.85-5.24)	3.78 (3.17-4.52)
Control 5	7.87 (7.36-8.42)	8.83 (8.18-9.53)	4.95 (4.48-5.47)	4.70 (4.18-5.28)	3.76 (3.47-4.08)	5.39 (4.86-5.98)
<i>M. duytsi</i>	7.63 (6.43-9.05)	9.12 (8.56-9.72)	4.59 (3.98-5.29)	4.65 (4.39-4.92)	3.72 (2.79-4.95)	5.66 (5.32-6.02)
<i>M. maritima</i>	7.53 (7.07-8.02)	8.72 (8.03-9.47)	5.08 (4.37-5.90)	4.69 (4.44-4.96)	4.20 (3.26-5.42)	5.33 (4.89-5.80)
<i>Pratylenchus spp.</i>	7.16 (6.45-7.95)	8.59 (7.82-9.44)	4.83 (4.09-5.70)	4.73 (4.61-4.85)	4.15 (3.68-4.69)	5.61 (5.32-5.91)
<b>Rhizome</b>						
Control 1	0.37 (0.07-2.01)		0	0.36 (0.14-0.92)	1.97 (1.65-2.34)	2.10 (1.46-3.02)
<i>H. pseudorobustus</i>	0.43 (0.26-0.74)		0	0.81 (0.50-1.30)	2.05 (1.60-2.62)	1.76 (1.03-3.00)
<i>H. conida</i>	0.57 (0.38-0.84)		0	0.81 (0.48-1.36)	1.79 (1.48-2.16)	1.33 (0.33-5.28)
<i>M. xenoplax</i>	0.20 (0.04-0.93)		0	0.75 (0.47-1.19)	1.70 (1.43-2.03)	1.97 (1.42-2.72)
<i>R. goodeyi</i>		0.52	0	0.93 (0.64-1.34)	1.76 (1.56-1.98)	1.19 (0.44-3.18)
Control 2			0	0.26 (0.21-0.31)	0.81 (0.62-1.07)	0.62 (0.27-1.43)
<i>N. microphasmis</i> <sup>a</sup>	0.68 (0.22-2.07)		0	0.14 (0.07-0.28)	0.70 (0.49-1.00)	0.76 (0.50-1.15)
Control 3			0	0.17 (0.08-0.34)	0.28 (0.20-0.39)	0.27 (0.11-0.64)
<i>T. ventralis</i> <sup>a</sup>	0.83 (0.47-1.48)		0	0.14 (0.09-0.23)	0.28 (0.16-0.51)	<b>0.08 (0.03-0.20)</b>
						<b>0.06 (0.02-0.21)</b>

Control 5	0.68 (0.02-19.2)	0	1.37 (1.15-1.62)	2.66 (2.30-3.09)	3.75 (3.02-4.67)	1.79 (1.22-2.62)
<i>M. duytisi</i>	0.29 (0.20-0.40)	0	1.34 (0.90-1.99)	2.95 (2.70-3.22)	2.58 (1.19-5.56)	2.21 (1.91-2.57)
<i>M. maritima</i>	0.47 (0.18-1.25)	0	1.38 (0.98-1.94)	2.81 (2.40-3.30)	2.44 (1.41-4.21)	1.51 (0.91-2.49)
<i>Pratylenchus spp.</i>	0.64 (0.23-1.82)	0	1.02 (0.59-1.75)	2.61 (2.13-3.20)	2.75 (1.81-4.17)	1.78 (1.25-2.54)
Root						
Control 1	2.31 (1.92-2.77)	3.22 (2.43-4.26)	3.75 (3.09-4.55)	4.79 (3.79-6.05)	4.76 (3.81-5.95)	3.92 (2.29-6.72)
<i>H. pseudorobustus</i>	2.00 (1.26-3.18)	2.63 (2.09-3.31)	3.39 (2.65-4.34)	3.86 (3.12-4.79)	4.76 (3.90-5.82)	3.50 (3.30-3.71)
<i>H. conida</i>	2.11 (1.80-2.47)	<b>2.16 (1.68-2.78)</b>	3.21 (3.00-3.43)	3.88 (3.36-4.47)	4.52 (3.65-5.60)	3.55 (2.99-4.23)
<i>M. xenoplax</i>	2.62 (2.19-3.13)	2.78 (2.57-3.01)	2.89 (2.20-3.80)	3.66 (3.09-4.33)	4.59 (4.03-5.22)	3.78 (3.33-4.28)
<i>R. goodeyi</i>	2.27 (1.93-2.67)	2.46 (2.31-2.63)	3.14 (2.68-3.68)	3.59 (2.98-4.32)	5.09 (4.11-6.30)	3.15 (2.67-3.72)
Control 2		1.25 (0.97-1.61)	2.37 (2.10-2.69)	2.05 (1.43-2.94)	5.92 (3.62-9.70)	3.85 (2.16-6.88)
<i>N. microphasmis</i> <sup>a</sup>	2.25 (1.79-2.83)	1.40 (0.83-2.35)	1.93 (1.47-2.54)	1.69 (1.16-2.47)	4.28 (3.11-5.90)	2.43 (1.91-3.10)
Control 3		0.37 (0.27-0.50)	0.79 (0.51-1.23)	0.99 (0.65-1.49)	3.93 (2.64-5.85)	1.23 (0.93-1.64)
<i>T. ventralis</i> <sup>a</sup>	<b>1.42 (1.29-1.56)</b>	<b>0.15 (0.09-0.25)</b>	<b>0.28 (0.13-0.58)</b>	0.70 (0.56-0.88)	2.63 (2.07-3.35)	<b>0.36 (0.28-0.45)</b>
Control 4 <sup>b</sup>	2.54 (2.02-2.65)	0.54 (0.49-0.82)	4.65 (2.48-7.42)	n.d.	6.21 (5.37-6.74)	4.71 (3.48-4.81)
<i>H. arenaria</i> <sup>b</sup>	<b>0.83 (0.78-1.03)</b>	0.62 (0.53-0.68)	<b>1.99 (1.80-2.23)</b>	n.d.	<b>4.29 (3.41-4.90)</b>	3.90 (2.49-4.49)
Control 5	2.12 (1.61-2.79)	1.81 (1.49-2.20)	2.37 (1.66-3.40)	2.03 (1.94-2.13)	3.82 (3.51-4.17)	3.62 (2.95-4.43)
<i>Pratylenchus spp.</i>	2.25 (2.01-2.52)	2.01 (1.55-2.59)	2.61 (1.88-3.61)	1.83 (1.48-2.27)	4.58 (3.78-5.55)	3.67 (2.48-5.42)

<sup>a</sup> The experiment with addition of *N. microphasmis* and *T. ventralis* to *E. juncea* was performed at the same time as control 1. Therefore, *E. juncea* was excluded from the comparison among plants.

<sup>b</sup> Rhizome and root were not separated when testing *H. arenaria*. Because a non-parametric test was used for this nematode species, median instead of mean values are shown.

N.d.: not determined.

Table 5. Results of two-way ANOVA (F-value) or Scheirer-Ray-Hare (H-value; roots of plants inoculated with *H. arenaria*) testing the effect of addition of nematodes (yes/no) on shoot, rhizome and root biomass of six plant species (see Table 4). Significant effects are presented in bold.

Plant part Nematode species	Plant species			Nematode addition			Plant × Nematode			Error	
	df	F/H	p	df	F/H	p	df	F/H	p	df	MS
<b>Shoot</b>											
<i>H. pseudorobustus</i>	5	40.46	< <b>0.001</b>	1	1.12	0.296	5	0.35	0.879	48	0.0136
<i>H. conida</i>	5	40.72	< <b>0.001</b>	1	2.22	0.142	5	0.64	0.673	48	0.0148
<i>M. xenoplax</i>	5	52.11	< <b>0.001</b>	1	0.06	0.812	5	1.38	0.249	48	0.0129
<i>R. goodeyi</i>	5	48.50	< <b>0.001</b>	1	0.01	0.904	5	2.49	<b>0.044</b>	48	0.0132
<i>N. microphasmis</i>	4	19.80	< <b>0.001</b>	1	3.58	0.066	4	0.67	0.619	40	0.0089
<i>T. ventralis</i>	4	19.44	< <b>0.001</b>	1	22.99	< <b>0.001</b>	4	4.62	<b>0.004</b>	40	0.0421
<i>H. arenaria</i>	4	21.02	< <b>0.001</b>	1	1.51	0.226	4	0.52	0.720	47	0.0208
<i>M. duytsi</i> <sup>a</sup>	5	104.70	< <b>0.001</b>	1	0.10	0.751	5	0.48	0.788	48	0.0105
<i>M. maritima</i> <sup>a</sup>	5	107.55	< <b>0.001</b>	1	0.20	0.656	5	0.82	0.539	48	0.0086
<i>Pratylenchus spp.</i>	5	145.21	< <b>0.001</b>	1	0.00	0.976	5	1.71	0.150	47	0.0060
<b>Rhizome</b>											
<i>H. pseudorobustus</i>	4	15.40	< <b>0.001</b>	1	1.88	0.178	4	0.90	0.475	40	0.3777
<i>H. conida</i>	4	9.21	< <b>0.001</b>	1	0.62	0.437	4	1.26	0.303	40	0.4733
<i>M. xenoplax</i>	4	17.77	< <b>0.001</b>	1	0.03	0.874	4	1.40	0.253	38	0.3735
<i>R. goodeyi</i>	4	7.99	< <b>0.001</b>	1	0.76	0.388	4	1.77	0.155	37	0.4397
<i>N. microphasmis</i>	3	17.03	< <b>0.001</b>	1	2.44	0.129	3	1.21	0.321	31	0.2507
<i>T. ventralis</i>	3	3.82	<b>0.019</b>	1	8.75	<b>0.006</b>	3	2.35	0.091	32	0.4076
<i>M. duytsi</i>	4	29.84	< <b>0.001</b>	1	2.41	0.129	4	2.31	0.075	38	0.1731
<i>M. maritima</i>	4	16.90	< <b>0.001</b>	1	1.70	0.201	4	0.50	0.735	38	0.2250
<i>Pratylenchus spp.</i>	4	14.84	< <b>0.001</b>	1	0.93	0.341	4	0.24	0.915	37	0.2298
<b>Root</b>											
<i>H. pseudorobustus</i>	5	15.84	< <b>0.001</b>	1	4.82	<b>0.033</b>	5	0.30	0.912	48	0.0519
<i>H. conida</i>	5	20.97	< <b>0.001</b>	1	10.74	<b>0.002</b>	5	1.02	0.418	48	0.0395
<i>M. xenoplax</i>	5	14.34	< <b>0.001</b>	1	4.19	<b>0.046</b>	5	1.51	0.205	48	0.0387
<i>R. goodeyi</i>	5	19.34	< <b>0.001</b>	1	8.83	<b>0.005</b>	5	1.37	0.254	48	0.0382
<i>N. microphasmis</i>	4	27.91	< <b>0.001</b>	1	6.20	<b>0.017</b>	4	1.19	0.329	40	0.0931
<i>T. ventralis</i>	4	84.27	< <b>0.001</b>	1	70.54	< <b>0.001</b>	4	3.63	<b>0.013</b>	40	0.1093
<i>H. arenaria</i> <sup>b</sup>	4	40.47	< <b>0.001</b>	1	4.16	< <b>0.05</b>	4	2.44	n.s.	-	273.37
<i>Pratylenchus spp.</i>	5	26.97	< <b>0.001</b>	1	1.25	0.269	5	0.57	0.723	47	0.0394

<sup>a</sup> Root biomass of the plants inoculated with *Meloidogyne* spp. was not determined.

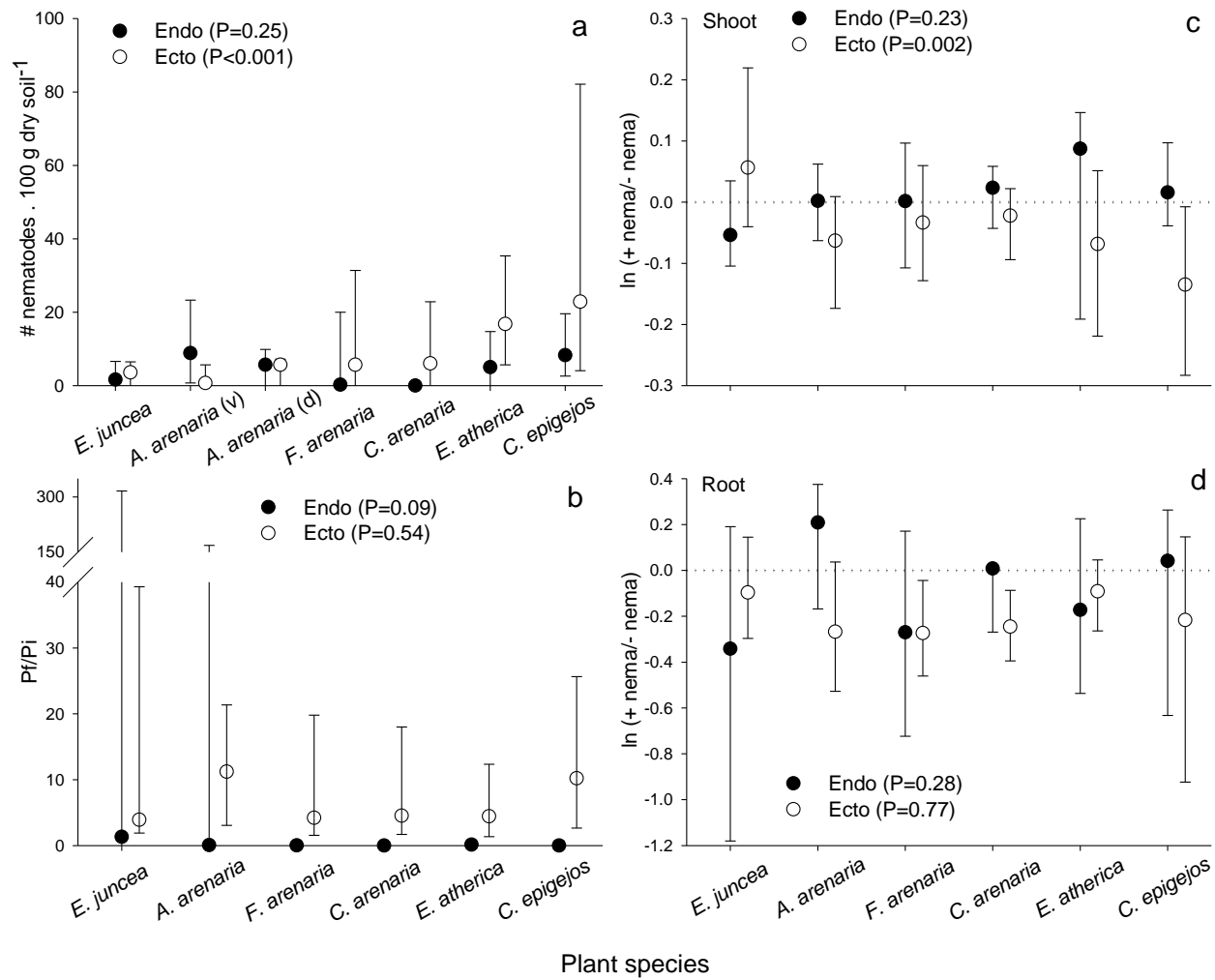
<sup>b</sup> Rhizome and root were not separated when testing this nematode species. H-values of non-parametric Scheirer-Ray-Hare test.

#### Figure captions

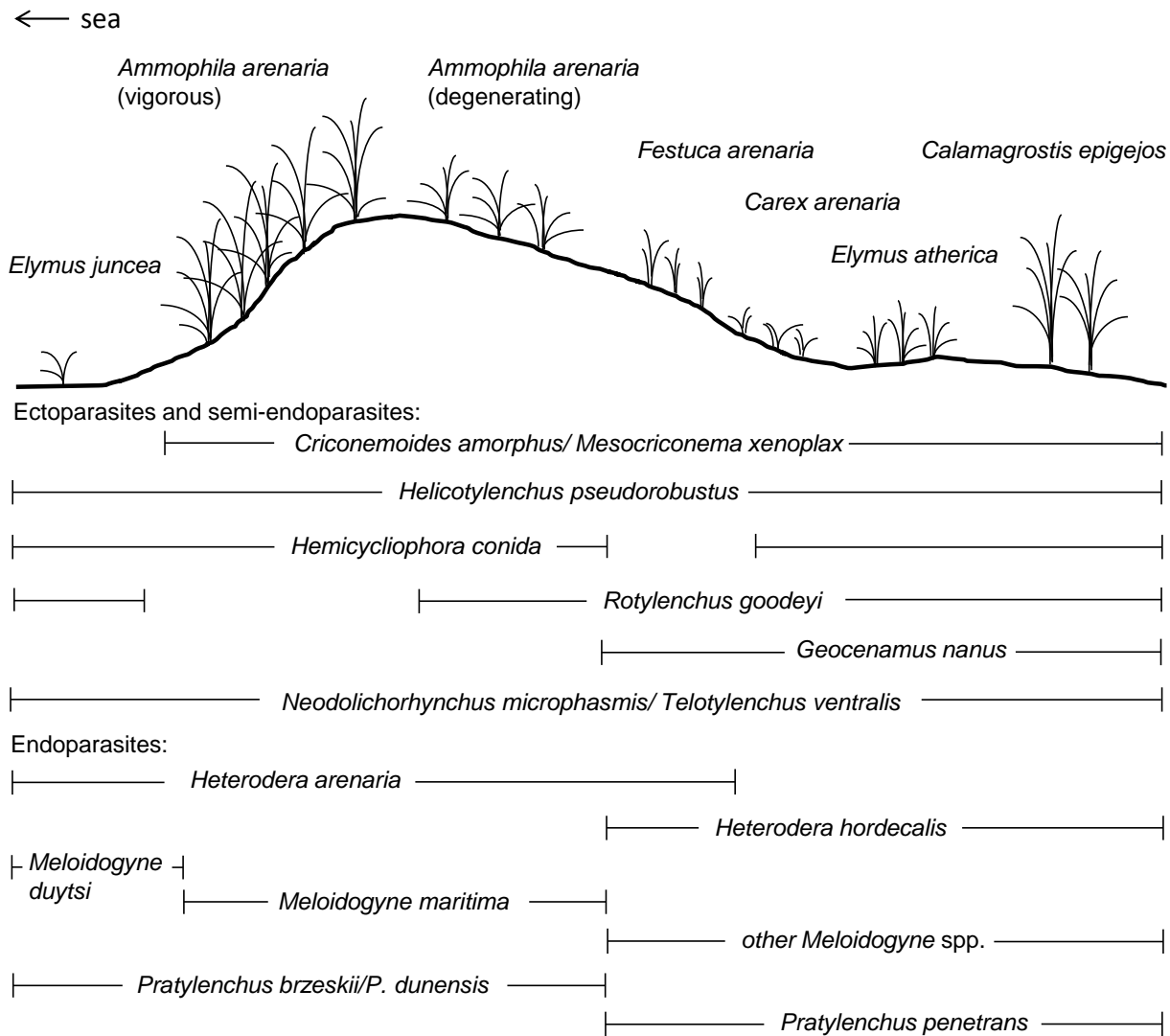
**Fig. 1** a) Field density (Number of nematodes  $\cdot$  100 g dry soil<sup>-1</sup>) of endoparasites and ecto- and semi-endoparasites that occurred with dominant plant species across a successional gradient in coastal dunes from the sea (left) land inward (right; n=16); (v) and (d) indicate vigorous and degenerated stages of *A. arenaria*, respectively. b) The ratio of final to initial population size (Pf/Pi) of endoparasites (only *Pratylenchus* spp. and *Meloidogyne* spp.) and ecto- and semi-endoparasites when added to the same plant species and c) effect of nematode addition on shoot and d) root biomass of the plants (ln( +nema/-nema); n=5, except for *H. arenaria* n=6). Median  $\pm$  quartile values are presented in all four panels; note the breaks on the y-axes of panels a) and b). P-values indicate significance of Spearman's rank-order correlations.

**Fig. 2** Occurrence of plant-feeding nematode species with dominant plant species across a successional gradient in coastal sand dunes, ranging from the sea (left) to landward (right), based on an overview of literature and own experience (see text).





**Fig. 1**



**Fig. 2**

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