

## Impact of native plant-parasitic nematode communities on the establishment of Meloidogyne chitwoodi

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1	Impact of native plant-parasitic nematode communities on the establishment of Meloidogyne
2	chitwoodi.
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12	Running head: PPNs competition with <i>M. chitwoodi</i>
13	
14	Abstract
15	Among plant-parasitic nematodes (PPNs), the species Meloidogyne chitwoodi constitutes an
16	important agricultural issue and is listed as a quarantine species in the EU. Here we investigate
17	whether PPN communities can affect the establishment and expansion of <i>M. chitwoodi</i> during a
18	simulated introduction. We developed an original experimental design on potato in glasshouse
19	involving four PPN communities derived from a single natural one that was initially grown on
20	different host plants. M. chitwoodi was inoculated in two different densities (50 or 1000 second-
21	stage juveniles (J2) per pot). After four months, <i>M. chitwoodi</i> was more abundant in the community
22	showing a low overall abundance of PPNs, decreased in the other PPN communities and failed to
23	establish in five pots out of 80. Results showed that establishment - even starting from a very low
24	inoculum - can occur in all communities even if indigenous PPNs in the community affect the
25	expansion through interspecific competition. The reverse interaction was also observed as PPN taxa

26 were generally less abundant in the 1000 *M. chitwoodi* J2 inoculation treatments. The proportion of

produced *M. chitwoodi* males was higher in the community showing low overall PPN abundance
suggesting a strong sensitivity of *M. chitwoodi* to intraspecific competition. The presented results
suggest that a low abundance of indigenous PPNs present a higher risk of expansion of *M. chitwoodi*even if the introduced inoculum is low.

32 Keywords

- 33 Competition phenomenon, original experimental design, plant-parasitic nematode communities,
- 34 potato, quarantine species

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Plant-parasitic nematodes (PPNs) are widely distributed organisms that feed on plant tissues and 38 can cause severe damage to crops (Bridge & Starr, 2007). Among PPNs, root-knot nematodes 39 40 (Meloidogyne spp.) can lead to high economic losses. These endoparasitic organisms have an 41 extremely wide host range and can multiply profusely on numerous types of crop (Trudgill, 1997; 42 Trudgill & Blok, 2001), producing galls on roots and severely reducing yields (Bridge & Starr, 2007). 43 Meloidogyne chitwoodi is one of the most damaging species in Europe owing to its broad range of 44 host plants including many crops such as root crops, tomato, wheat or maize (O'Bannon et al., 1982; Karssen, 2002; EPPO bulletin, 2004). Furthermore, this species reproduces parthenogenetically 45 (Trudgill, 1997; Castagnone-Sereno, 2006), multiplying rapidly and massively through female 46 47 production only. Males are sometimes produced under adverse environmental conditions, such as poor food quality or competition (Trudgill, 1972; Triantaphyllou, 1973). In contrast to the tropical 48 49 root-knot nematode species that reproduces by obligate mitotic parthenogenesis (Castagnone-50 Sereno, 2006), M. chitwoodi reproduces by facultative meiotic parthenogenesis (Van der Beek & 51 Karssen, 1997); therefore, when males of *M. chitwoodi* are present in the populations, amphimixis 52 can occur (Castagnone-Sereno, 2006). This species has been classified as a quarantine species in the European Union and because it is considered as invasive, it has to be immediately contained upon 53 54 detection in a cultivated field. In France, this species was detected in only a few foci between 1996 55 and 2014: the Nord-Pas de Calais, Picardy, Lower Normandy, Brittany, Ile-de-France and Aquitaine 56 administrative regions (Gamon & Lenne, 2012). Upon detection, French legislation requires that 57 farmers practice bare fallow for at least one year. Published studies carried out on the management 58 of M. chitwoodi mainly focus on plant-nematode interactions, including nematode virulence and 59 plant resistance (van der Beek et al., 1998 ; Janssen et al., 1995; Brinkman et al., 1996; Jensen & 60 Griffin, 1997; Castagnone-Sereno, 2002) or plant diffusates (Wesemael et al., 2006; Khokon et al., 61 2009).

62 Other approaches, including preventive steps to assess and thus limit the risk of PPNs 63 establishment, are important to consider. A monitoring plan is carried out annually by French 64 authorities across the country in crop fields to detect *M. chitwoodi* introductions. However, these fields are defined as "at-risk" only on the basis of the host crop grown, and not according to the 65 66 existing native PPN community. During an invasion, the introduced species comes into interaction 67 with the organisms already present in the community and possible antagonistic interactions, such as competition, can occur and limit the establishment and/or multiplication of the introduced PPNs. 68 69 Three of the six categories of competition proposed by Schoener (1983), pre-emptive competition, 70 chemical competition and consumptive competition, are useful for summarising competitive 71 interactions among PPNs. Pre-emptive competition refers to ecological niche occupation by other 72 species, thereby preventing another species from entering that space. Chemical competition occurs 73 when a species produces a toxin or induces the production of allelochemicals that suppress another 74 species. Consumptive competition occurs when some essential resources, usually food, is consumed 75 by one species, reducing or depleting the quantity available to the competing species. Competition 76 (for example for access to resources or space) has already been reported between nematode species 77 (Freckman & Caswell, 1985; Umesh et al., 1994). Although other PPN species have already been 78 shown to have a negative impact on the development, multiplication rate or hatching of root-knot 79 nematodes (Diez et al., 2003; Brinkman et al., 2008), few studies have explored M. chitwoodi 80 interactions with other PPNs. For instance, (Umesh et al., 1994) observed that Pratylenchus neglectus 81 can limit *M. chitwoodi* growth or hatching in barley if it penetrates the roots first. Similar studies have been conducted in more complex systems involving other organisms (Al-Rehiayani et al., 1999) 82 83 and have highlighted three-way interactions between a bacteria (Bacillus megaterium), a PPN 84 (Pratylenchus neglectus) and green manure on the development of M. chitwoodi. Various types of 85 competition can be observed depending on the feeding habit; endoparasitic nematode species likely 86 compete mainly for space in the roots and ectoparasitic species for access to the resource (Umesh et 87 al., 1994).

In this study, we focused on PPN community interactions with *M. chitwoodi* to assess the potential of these communities for limiting *M. chitwoodi* multiplication or even its establishment after introduction. We modified a natural field community by using different host plants to obtain four contrasted PPN communities with the same taxonomic richness but varying in global and relative abundance of PPNs or feeding type (*i.e.* ectoparasite *vs* endoparasite) and tested the impact of these PPN communities on the multiplication rate of *M. chitwoodi*.

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#### 95 Materials and Methods

#### 96 Initial community and preliminary experiment

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98 To test and assess the effect of various types of PPN community but minimise the heterogeneity 99 of other variables between the communities (such as soil properties or history of cultural practices), 100 an initial PPN community was sampled in a field free of root-knot nematodes near the French 101 National Institute for Agricultural Research in Brittany (48°06'30"N 1°47'34.0"W). PPNs were 102 extracted from 400 g of soil previously homogenised according to EPPO bulletin PM 7/119 part 3.4 103 (2013) for morphological identification at the genus level when possible. The soil was mixed with 1/3 104 of PPN-free sand to give a texture allowing better plant growth and nematode movements and 105 extraction. Nineteen 22 L pots were filled with this soil and planted with either wheat (three pots), 106 maize (four pots), sugar beet (four pots), carrot (three pots) or potato (three pots). Two additional 107 pots were left free of any host plant. We let the initial PPN community develop in the different pots 108 for four months (25°C, L:D 12:8 and watered twice a week) to manipulate the abundance of PPN 109 taxa. After four months, PPNs were extracted and counted in 400 g of homogenised soil from each 110 pot according to PM 7/119 part 3.4 (2013) and endoparasitic nematodes (i.e. Pratylenchus) were 111 extracted from maize and carrot roots using a mist chamber. Those two plants were chosen as they 112 are known to be good host plants for *Pratylenchus* (Bridge & Starr, 2007).

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116 From the preliminary experiment, we obtained contrasted communities (Table 1). Soil from wheat 117 and potato pots was not used in further experiments in order to keep only the most different 118 communities in terms of either total abundance or endoparasite vs ectoparasite abundance. The soil 119 from each pot was thoroughly mixed, homogenised and distributed to 106 new individual 2.8 kg pots 120 for the main experiment. Soil from maize, enriched with 150 Pratylenchus per pot, was used for 121 modality 1 (HT). Soil from uncultivated pots was used for modality 2 (LT). Soil from carrot, enriched 122 with 1500 Pratylenchus per pot, was used for modality 3 (HEN). Soil from sugar beet was used for 123 modality 4 (HEC). Control 3 (C3, Table 1) was obtained by mixing all the previous soils (without 124 Pratylenchus enrichment) to create the average community. PPN-free soil, from the same field but 125 stored several years in a container, also mixed with 1/3 of PPN-free sand was used for Controls 1 and 126 2 (C1 and C2, Table 1). Pots were planted with one potato tuber (var. Désirée) and distributed 127 randomly in the greenhouse. M. chitwoodi (population 13-765 from Lower-Normandy) was 128 inoculated in each pot 10 days after planting, in three spots around the potato tuber, near the roots. 129 This population was previously reared on tomato in the greenhouse and is part of the ANSES - Plant 130 Health Laboratory collection. To obtain second-stage juveniles (J2) for inoculation, roots harbouring 131 egg masses were placed in a mist chamber until hatching. For each community type and C2, 10 pots 132 were inoculated with 1000 M. chitwoodi J2 and 10 other pots with 50 J2. Three replicates of both C1 133 and C3 were also prepared for a total of 106 individual pots. We ran this experiment for one potato 134 growth cycle (about four months) at 25°C, 12 hours photoperiod and watered twice a week. At the 135 end of the experiment, above-ground plant parts were eliminated. PPNs were extracted again from 136 400 g of homogenised soil from each pot. Roots and tubers were separated, weighed and digested 137 overnight according to PM 7/119 part 2.7 (2013). PPNs were then recovered from the enzymatic digestion by centrifugal flotation (PM 7/119 part 3.5, 2013). PPNs from the three extracts (soil, 138 139 tubers and roots) of each pot were identified based on morphological criteria using a

stereomicroscope. For standardisation, each taxon was counted, after a dilution step (depending on the PPNs concentration) in 5 mL of the dilution. Only *M. chitwoodi* males and females (J4 and adults) were counted separately in each extracts to obtain information on the proportion of inoculated J2 that reached the adult stage. *M. chitwoodi* J2 (J2 and J3) were not counted as we were not able to distinguish those inoculated that have not reach the adult stage for any reason from those of the following generations. For all the other taxa, all the developmental stages were counted.

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#### 147 Statistical analyses

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The growth rate of each PPN taxon was calculated based on the initial density using the following equation: (Pf – Pi) / Pi, where Pi is the initial population density of each taxon and Pf is the final population density of each taxon at the end of the experiment. For the growth rate, no distinction was made between *M. chitwoodi* females and males because undifferentiated J2 were used for the inoculations.

154 All statistical analyses were conducted using R software (R Core Team, 2016). Comparisons 155 between taxon abundances and growth rate across the communities were performed using 156 generalised linear models (GLMs). We used Poisson models for abundance data and Gaussian model 157 for growth rate and percentage data. Multiple comparisons between community types were 158 performed using the estimated marginal means (EMMeans) method with the emmeans R package 159 (Lenth, 2018). Comparisons between C2 and all the other modalities for abundance and growth rate 160 of *M. chitwoodi* were performed first (results are presented in Figure S1 and Figure S2). It appeared 161 that C2 was always significantly different from the other modalities (except considering growth rate, 162 when C2 was compared to LT in pots inoculated with 1000 J2). Thus, comparisons between 163 modalities, without C2, were then performed in order to highlight more precisely the variation.

165 Results

#### 166 **Preliminary experiment**

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Five PPN taxa were identified in the initial community from the sampled field: Pratylenchus, 168 169 Helicotylenchus, Telotylenchidae, Paratylenchus and Criconemoïdes. Table 2 shows the abundance of 170 each PPN taxon before the preliminary experiment and after the development in the different pots 171 containing different host plants. Most importantly, Paratylenchus (ectoparasite) reproduced 172 extremely well on sugar beet (1960 ± 183 individuals per 400 g of soil) compared with the other host 173 plants and *Pratylenchus* (endoparasite) was recovered in high quantity (12139 ± 4086 individuals) from the roots of maize and used to adjust the modalities HT and HEN. Soils from bare soil, maize, 174 175 sugar beet and carrot were used to design four modalities (HT to HEC, Table 1) as described in the 176 Material and Method section.

177

#### 178 Impact of PPN communities on *M. chitwoodi* populations

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180 After 4 months of potato culture using the modalities previously designed, abundance of M. 181 chitwoodi was determined in all pots. M. chitwoodi abundance was limited by the other PPNs 182 because its total abundance was significantly lower in all the modalities than in the control 183 containing M. chitwoodi alone on potato (Figure S1), regardless the inoculation treatment (609.50 ± 184 73.88 in pots inoculated with 50 J2 and 1009.73  $\pm$  127.73 in pots inoculated with 1000 J2). Figure 1 185 shows comparison of M. chitwoodi abundances between modalities without the control. M. 186 chitwoodi females and males followed similar abundance patterns, although males were always less 187 abundant than females (Figure 1). They showed their highest abundance in LT (164.00 ± 37.49 and 188 742.80 ± 86.93 females per pot, in the 50 and 1000 J2 inoculation treatment, respectively, and 83.28 ± 23.79 and 170.35 ± 17.58 males per pot, in the 50 and 1000 J2 inoculation treatment, respectively) 189 190 (Figure 1). None of the pots inoculated with 1000 M. chitwoodi J2 was found free of M. chitwoodi at

the end of the experiment. Only four pots out of the 10 for HT and 1 pot out of the 10 for HEN inoculated with 50 J2 were found free of *M. chitwoodi* adults at the end of the experiment. Other pots inoculated with 50 *M. chitwoodi* J2 showed mean final abundance of  $12 \pm 3.12$ ,  $247 \pm 54.44$ ,  $8 \pm$ 2.72 and  $59 \pm 23.64$  for HT, LT, HEN and HEC respectively.

195

196 In order to understand the variations of abundance observed, we also compared the growth rate 197 of each taxon among the communities using Gaussian models. Results about M. chitwoodi are given 198 in Figure 2. *M. chitwoodi* multiplication was significantly more important in the control (mean C2 = 199 11.19  $\pm$  1.48) than in the other modalities for pots inoculated with 50 J2 but did not vary from 200 multiplication in LT for pots inoculated with 1000 J2 (Figure S2). In HT, HEN and HEC, M. chitwoodi 201 populations showed a decrease (Figure 2a). Significant difference between inoculation treatments 202 among each modality appeared only for LT ( $3.95 \pm 1.05$  for pots inoculated with 50 J2 compare to -203  $0.09 \pm 0.09$ ) but not for the other modalities (Figure 2b). However, it should also be noticed that 204 while growth rate values differ drastically among the modalities in the 50 J2 inoculum, these values 205 appear to vary in a much narrower range in the 1000 J2 inoculum. This suggests that the strength of 206 the effect of the community on *M. chitwoodi* growth rate depends also of the invasive inoculum size.

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#### Effect of *M. chitwoodi* densities on other PPN

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210 Comparisons between final abundance of PPN in the two inoculation treatments among the 211 modalities are presented in Figure 3. Significantly higher abundance in 50 J2 inoculation treatment 212 than in 1000 J2 inoculation treatment was observed in HT, HEN and HEC for *Pratylenchus*, in LT and 213 HEC for *Helicotylenchus*, in HT, LT and HEN for *Paratylenchus*, in LT and HEC for Telotylenchidae and 214 in HT and LT for *Criconemoïdes*. On the contrary, significantly higher abundance in 1000 J2 215 inoculation treatment than in 50 J2 inoculation treatment was observed in a much more limited number of situations: only in HT for *Helicotylenchus*, only in HEC for *Paratylenchus*, in HT and HEN for
Telotylenchidae and only in HEC for *Criconemoïdes*.

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219 For each PPN taxon, growth rate was compared between modalities in pots inoculated with 50 220 and 1000 M. chitwoodi J2 (Figure 4a) and between inoculation treatments within each modality 221 (Figure 4b). It appears that for Paratylenchus and Criconemoïdes, a negative growth rate was 222 observed in all the modalities and no significant difference was observed between inoculation 223 treatments (Figure 4). There was globally no significant difference in PPN growth rate between the 224 M. chitwoodi inoculation treatments. In only two cases (i.e. Pratylenchus HEC and Helicotylenchus LT) 225 a significant difference appeared between the M. chitwoodi inoculation treatments and there was 226 always a more important multiplication in the 50 J2 inoculation treatment than in the 1000 J2 227 inoculation treatment (Figure 4b).

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#### 229 Proportion of M. chitwoodi males

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To further investigate the observations about *M. chitwoodi* abundance, we studied the proportions of males among communities and inoculation treatments using Gaussian models (Figure 5). Interestingly, males were more abundant in LT compare to the other modalities regardless the inoculation treatment (Figure 5a). Furthermore, in LT, the percentage of males was higher in the 50 J2 inoculation treatment than in the 1000 J2 inoculation treatment (35.82% ± 5.28% vs. 19.70% ± 3.82%, respectively) (Figure 5b).

237

#### 238 Discussion

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This study highlighted for the first time the impact of natural PPN communities on *M. chitwoodi*. We choose to count only adults of *M. chitwoodi*, firstly because it was interesting to know the 242 abundance of J2 that have reached the adult stage and thus possibly multiplied, to study the 243 establishment of M. chitwoodi. Secondly, since at least two generations of M. chitwoodi occurred, it 244 was not possible to distinguish the J2 from the first generation (inoculated J2) which would not have 245 further developed into adults from the J2 of the second or even third generations. Considering this, 246 final abundance and growth rate based on adults bring a relevant information about the impact of 247 PPN communities on M. chitwoodi establishment but as J2 were present in the modalities, the 248 abundance figures presented in this study were slightly underestimated but much more comparable 249 among each other.

250 We created different community types from a single community to limit variation in variables other than PPN community composition. Although abiotic factors were fully controlled during our 251 252 experiment, we cannot exclude the possibility that biotic factors other than PPNs (such as 253 microorganisms or non-phytoparasitic nematodes that were present in our soils) are also involved 254 and contribute to the observed decrease of M. chitwoodi (Akhtar & Malik, 2000). However, the 255 potential of the initial soil loading for microorganisms other than PPNs was the same because each 256 modality was established on the basis of the same soil and the experiment was conducted over a 4-257 month period and only using potato. Therefore questions remain: would the observed decrease in M. 258 chitwoodi continue over a longer period and successive cropping or would M. chitwoodi ultimately 259 adapt to its competitors? Would similar results be observed using the same community types but 260 different host plants? Additional experiments will provide more information and should be now 261 considered to confirm these first results.

262

The abundance of *M. chitwoodi* greatly decreases during a potato cycle in three modalities (HT, HEN and HEC) which corresponded to a high overall abundance of indigenous PPNs, irrespective of their feeding habit (endoparasite *vs.* ectoparasite). Similar results were observed for *Meloidogyne maritima* whose development is reduced by *Pratylenchus penetrans* and *Heterodera arenaria* in a PPN community on dune grass (Brinkman *et al.*, 2005). Furthermore, dune grass biomass is lower

268 when *M. maritima* is added alone to the plant than when all three species are present. The two latter 269 PPN species may "protect" their host plant by delaying the development of *M. maritima*, which then 270 occurs later in the growing season when infection by *M. maritima* has less impact on the plant 271 (Brinkman et al., 2005). However, it is unlikely that such effect occur in our study because the fresh 272 weight of roots and tubers did not significantly vary between the three controls: C1 (potato alone), 273 C2 (M. chitwoodi alone) and C3 (average PPN community) (data not shown). Instead, our results 274 appear to rather support interspecific competition between the native PPN communities and the 275 introduced species for access to roots, with the PPNs already present in soil colonising the roots first 276 and preventing *M. chitwoodi* from penetrating them and achieve its development. However, if the 277 PPNs abundance is low, the invasive species seems to have enough room to penetrate and multiply 278 among roots and tubers.

279 Overall abundance of PPN in HEN was however lower than in HT and HEC (in pots inoculated with 280 50 M. chitwoodi J2 for instance, HEN total PPN abundance was 267 ± 55 whereas HT and HEC total 281 abundance were respectively 1201 ± 138 and 946 ± 160). In this modality in particular, relative 282 abundance of Pratylenchus was higher than in the other, and could highlight an impact of this taxon 283 on M. chitwoodi as it has been observed in Umesh et al. (1994). Even if we cannot exclude the 284 competitor effect of species of Pratylenchus towards M. chitwoodi, our results highlight the impact of 285 the overall abundance of the PPN community as failure of establishment of M. chitwoodi was 286 observed predominantly in the HT community type and not the HEN community type. It should also 287 be emphasized that such failure of establishment was only observed for low invasive inoculum size 288 (i.e. 50 J2). In natural agrosystems, this number could for example be due to soil transfer through 289 agricultural machinery (highlighted for cyst nematodes in Plantard et al. (2008)) or contaminated 290 seedling tubers.

Observed *M. chitwoodi* growth rate variations between the modalities appear to confirm the abundance results. We observed around a five-fold increase in *M. chitwoodi* in LT pots inoculated with 50 J2 compared with the other modalities for which the *M. chitwoodi* population has decreased,

even until elimination in few pots. This confirms the existence of strong interspecific competitionthat can be useful to prevent establishment when the invasive inoculum is low.

296

297 To simulate an indigenous PPN community which is already present and established in a field, M. 298 chitwoodi inoculations were performed 10 days after planting potato tubers to allow other PPNs to 299 colonise the roots first. Despite the observed decrease of the quarantine species, an inverse effect 300 was also noticed on the other PPN taxa. Except in LT, abundance of Pratylenchus for example was 301 lower in the 1000 J2 inoculation treatment than in the 50 J2 inoculation treatment despite the 302 delayed M. chitwoodi inoculation. Similar results were observed for the other PPN taxa present in 303 our study. This type of inverse effect has already been observed in previous studies (Villenave & 304 Cadet, 1998; Melakeberhan & Dey, 2003), suggesting reciprocal interspecific competition between 305 the indigenous PPNs and the invasive species. Furthermore, growth rates of these taxa were either 306 not significantly different or lower in the 1000 J2 inoculation treatment than in the 50 J2 inoculation 307 treatment. However, it should be noticed that Paratylenchus and Criconemoïdes populations have 308 decreased in all modalities, likely due to poor host (i.e. potato) suitability (Nickle, 1991; Bridge & 309 Starr, 2007) rather than to a direct effect of M. chitwoodi on these taxa. Accordingly, in the 310 preliminary experiment, Paratylenchus and Criconemoïdes have not well multiplied on potato.

311 Our results therefore support previous findings showing effects of endoparasitic nematodes (*i.e.* 312 M. chitwoodi) on the development of ectoparasitic nematodes (such as Helicotylenchus or 313 Telotylenchidae) (Brinkman et al., 2004), as well as on other endoparasitic nematodes such as 314 Pratylenchus (Lasserre et al., 1994; Melakeberhan & Dey, 2003; Brinkman et al., 2008). We 315 hypothesise that the observed inverse effect is mainly due to interspecific competition for access to 316 roots and/or food, because previous results on Pratylenchus and M. chitwoodi have failed to show 317 any indirect competition effect by stimulating root defences (Umesh et al., 1994; Brinkman et al., 318 2008).

The decrease in the growth rate for the indigenous taxa appeared to be due to *M. chitwoodi* density because at least one of the community types showed significant differences between the *M. chitwoodi* inoculation treatments. However, we also sometime observed that the growth rates for PPN taxa were higher in LT - lowest overall PPN abundance - suggesting that PPN communities may regulate *M. chitwoodi* expansion and also partially regulate themselves through interspecific competition.

325

326 Interestingly, we observed unexpected variation in the male proportions among the community 327 types and inoculations. In the agriculturally important root-knot nematode species, reproduction is 328 generally parthenogenetic (Trudgill, 1997; Castagnone-Sereno, 2006). In some of these species, such 329 as M. chitwoodi, parthenogenesis is facultative. Hence, males can participate in reproduction 330 (Castagnone-Sereno, 2006). Although the sexual determinism of these facultative parthenogenetic 331 species is not fully understood, it nonetheless appears to be a phenomenon mainly linked to 332 environmental conditions (Castagnone-Sereno, 2006). Juvenile transformation is not pre-determined 333 and males are produced when the environment is not suitable for female development, for example 334 due to poor host quality, small size of the giant cells or strong competition (Triantaphyllou, 1973; 335 Trudgill, 1997). Juvenile differentiation thus depends on the amount of food consumed (Trudgill, 336 1972). Unexpectedly, we observed that the proportion of males was higher in LT than in the other 337 PPN community types, and among this modality, even higher in the 50 J2 inoculation treatment than 338 in the 1000 J2 inoculation treatment. This male production seems in contradiction with our other 339 results that highlight a high growth rate of *M. chitwoodi* in LT where the overall PPNs abundance was 340 low (i.e. lower interspecific competition). In cyst nematodes, which are also sedentary endoparasites 341 like root-knot nematodes, the production of males is attributed to heavy root infestation and thus 342 high intra-host density (Mugnéry and Phillips, 2007). Keeping in mind that two to even three 343 generations of *M. chitwoodi* took place during the time of our experiment, we hypothesize that 344 intraspecific competition occurred in the roots heavily infested with *M. chitwoodi* in LT by the end of

our experiment after an important multiplication. In this case, interspecific competition was weak and many *M. chitwoodi* J2 penetrated the roots to initiate feeding sites. It is therefore probable that those J2 became females and in turn produced many J2, explaining the high population growth rate. However, the second or third generation may have represented a threshold for the host plant which then produced inferior quality feeding sites due to heavy intraspecific competition, inducing the production of males.

351

352 This original experimental design allowed us to test the impact of different indigenous PPN 353 communities on the development and establishment success of M. chitwoodi. Clearly, soils showing a 354 poor PPN community in terms of abundance present a higher risk of successful establishment of M. 355 chitwoodi. It could be therefore interesting to consider this parameter in risk assessments and 356 monitoring plans in regard to this quarantine species. As discussed, the results presented in this 357 study need to be confirmed with other M. chitwoodi populations, but also other host plants and 358 longer experiments in order to propose a relevant risk assessment tool. Furthermore, studying the 359 impact of cultural practices on PPN communities in various agrosystems may allow identify the 360 practices that can help to maintain native and non-damaging PPN communities. This opens new 361 research areas for the management of nematode diversity in soils as a mean to regulate some soil-362 borne invasive species.

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365

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451

#### 452 Figures captions

453

Fig. 1: Mean final abundance (number of individuals per pot) of *M. chitwoodi* total adults, only females or only males, among the different modalities. Light grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error bars indicate standard error. Different letters indicate significant differences between modalities according to multiple comparison tests using the estimated marginal means method. Scale varies between graphs.

460

Fig. 2: (a) Growth rate of *M. chitwoodi* among the different modalities. (b) Comparison of *M. chitwoodi* growth rate between the inoculation treatments among each modality. Light grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error bars indicate standard error. Different letters indicate significant differences between modalities (a) or between inoculation treatments among the modalities (b) according to multiple comparison tests using the estimated marginal means method. Scale varies between graphs.

468

Fig. 3: Comparison of mean final abundance (number of individuals per pot) of each taxon between
the inoculation treatments among the modalities. Light grey indicate pots where *M. chitwoodi* J2
inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error

472 bars indicate standard error. Different letters indicate significant differences between inoculation 473 treatments among the modalities according to multiple comparison tests using the estimated 474 marginal means method. Dotted line indicates the mean abundance value of each PPN taxon in the 475 control pots (C3, see Table 1). Scale varies between graphs.

476

477 Fig. 4: (a) Growth rate of PPN taxa among the different modalities. (b) Growth rate comparison of 478 each PPN taxon between the inoculation treatments among each modality. Light grey indicate pots 479 where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 480 inoculum was 1000 J2. Error bars indicate standard error. Different letters indicate significant 481 differences between modalities (a) or between inoculation treatments among the modalities (b) 482 according to multiple comparison tests using the estimated marginal means method. Dotted line 483 indicates the mean abundance value of each PPN taxon in the control pots (C3, see Table 1). Scale 484 varies between graphs.

485

Fig. 5: (a) Percentage of *M. chitwoodi* males for each modality. (b) Comparison of *M. chitwoodi* males percentage between the inoculation treatments among each modality. Light grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error bars indicate standard error. Different letters indicate significant differences between modalities (a) or between inoculation treatments among the modalities (b) according to multiple comparison tests using the estimated marginal means method. Scale varies between graphs.

493

494 Fig. S1: Mean final abundance (number of individuals per pot) of *M. chitwoodi* total adults, only 495 females or only males, among the different modalities, including control pots where *M. chitwoodi* J2 496 were inoculated alone. Light grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark 497 grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error bars indicate standard error.

498 Different letters indicate significant differences between modalities according to multiple 499 comparison tests using the estimated marginal means method. Scale varies between graphs.

500

501 Fig. S2: (a) Growth rate of *M. chitwoodi* among the different modalities, including control pots where 502 M. chitwoodi J2 were inoculated alone. (b) Comparison of M. chitwoodi growth rate between the 503 inoculation treatments among each modality, including control pots where M. chitwoodi J2 were 504 inoculated alone. Light grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey 505 indicate pots where M. chitwoodi J2 inoculum was 1000 J2. Error bars indicate standard error. 506 Different letters indicate significant differences between modalities (a) or between inoculation 507 treatments among the modalities (b) according to multiple comparison tests using the estimated 508 marginal means method. Scale varies between graphs.

Treatment	Abbreviation	Characteristics
Control 1	C1	No PPNs
Control 2	C2	<i>M. chitwoodi</i> alone
Control 3	C3	Average PPN community composed of M1, M2, M3, M4 soil mixed together (prior to <i>Pratylenchus</i> inoculation), without <i>M. chitwoodi</i>
Modality 1	HT	High total abundance of PPNs (around 600 individuals per 400 g of soil, proportion of endo- and ectoparasites relatively similar)
Modality 2	LT	Low total abundance of PPNs (around 50 individuals per 400 g of soil, proportion of endo- and ectoparasites relatively similar)
Modality 3	HEN	High relative abundance of endoparasitic PPNs (around 65%, global abundance around 350 individuals per 400 g of soil)
Modality 4	HEC	High relative abundance of ectoparasites PPNs (around 98%, global abundance around 650 individuals per 400 g)

**Table 1** Characteristics of the community types used in the experiment

Host plant	Soil (individual	Roots (total individuals)				
	Pratylenchus	Helicotylenchus	Telotylenchidae	Paratylenchus	Criconemoïdes	Pratylenchus
Initial community	384	128	240	224	4	-
Bare soil	8 ± 3.00	7 ± 4.00	48 ± 5.50	29 ± 11.00	3 ± 0.50	-
Maize	62 ± 15.10	174 ± 17.09	97 ± 27.34	505 ± 95.39	$10 \pm 2.00$	12,139 ± 4085.72
Sugar beet	44 ± 5.16	60 ± 18.62	86 ± 18.29	1960 ± 182.87	6 ± 2.00	-
Wheat	73 ± 24.52	121 ± 24.01	516 ± 205.30	474 ± 222.43	11 ± 5.33	-
Carrot	12 ± 4.62	$11 \pm 3.48$	51 ± 7.17	117 ± 81.79	6 ± 2.03	107 ± 47.19
Potato	68 ± 29.41	28 ± 6.39	191 ± 62.78	73 ± 18.03	6 ± 2.85	-

**Table 2** Initial community and mean abundance ± standard error of each PPN taxon at the end of the preliminary experiment





M. chitwoodi females

# *M. chitwoodi* males 50 J2 inoculation



*M. chitwoodi* total 1000 J2 inoculation













Pratylenchus







Telotylenchidae



Criconemoïdes



Pratylenchus

LT

Helicotylenchus

а

LT

12

10

8

4

2

0

-2

45

40

35

30

25

20

15

10

5 0

ΗТ

Growth rate

h

ΗТ

Growth rate 6 1000 M. chitwoodi J2 inoculation

Pratylenchus

а

HEC

а

HEC

а

LT

а

LT

Helicotylenchus

HEN

b

Т

HEN

8

7

6

5

4

3

2

1

0

-1

25

20

15

10

5

0

b

ΗТ

b

ΗТ

а

HEC

b

HEC

b

HEN

с

HEN

(b) Comparison between M. chitwoodi J2 inoculum









0







Paratylenchus





Telotylenchidae





Telotylenchidae



Telotylenchidae







Criconemoïdes





#### M. chitwoodi total 50 J2 inoculation



M. chitwoodi females 50 J2 inoculation

#### 600 а 500 400 300 b 200 С 100 d 0 HEC ΗT LT HEN





M. chitwoodi total 1000 J2 inoculation



M. chitwoodi females 1000 J2 inoculation

С



M. chitwoodi males 1000 J2 inoculation



