Biogeographic differences in soil biota promote invasive grass response to nutrient addition relative to co-occurring species despite lack of belowground enemy release

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*Reasons for Highlighted Student Paper status:*

*In this greenhouse experiment we show that novel soil biota can increase the response of an invasive grass to nutrient additions relative to other species, even in the absence of belowground enemy release. This emphasises that abiotic and biotic global changes interact to facilitate species invasions.*

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**Abstract**

Multiple plant species invasions and increases in nutrient availability are pervasive drivers of global environmental change that often co-occur. Many plant invasion studies, however, focus on single-species or single-mechanism invasions, risking an oversimplification of a multifaceted process. Here we test how biogeographic differences in soil biota, such as belowground enemy release, interact with increases in nutrient availability to influence invasive plant growth. We conducted a greenhouse experiment using three co-occurring invasive grasses and one native grass. We grew species in live and sterilized soil from the invaders native (United Kingdom) and introduced (New Zealand) ranges with a nutrient addition treatment. We found no evidence for belowground enemy release. However, species' responses to nutrients varied, and this depended on soil origin and sterilization. In live soil from the introduced range the invasive species *Lolium perenne* L. responded more positively to nutrient addition than co-occurring invasive and native species. In contrast, in live soil from the native range and in sterilized soils, there were no differences in species' responses to nutrients. This suggests that the presence of soil biota from the introduced range allowed *L. perenne* to capture additional nutrients better than co-occurring species. Considering the globally widespread nature of anthropogenic nutrient additions to ecosystems, this effect could be contributing to a global homogenisation of flora and the associated losses in native species diversity.

## Introduction

Plant invasions are a pervasive driver of global environmental change (Vitousek et al. 1997; Sala 2000; Van Kleunen et al. 2015) and are associated with biodiversity loss (Vilà et al. 2011; Seabloom et al. 2015) and economic costs (Pimentel et al. 2005; Pejchar and Mooney 2009). At least 29 hypotheses have been proposed to explain invasive plant species success (Catford et al. 2009) indicating the inherent complexity of plant invasions. Despite a proliferation of biological invasion studies in recent decades (Richardson and Pysek 2008), many studies have focused on single species (Kuebbing et al. 2013) or mechanisms (Gurevitch et al. 2011). This risks oversimplifying a complex process as mechanisms are likely to interact (Blumenthal 2005; Blumenthal et al. 2009; Gurevitch et al. 2011; Maron et al. 2013) and vary for different co-occurring invasive species (Kuebbing et al. 2013). In addition, invasion may be facilitated by other, abiotic, environmental changes, such as increased resource availability via agricultural fertilisation, disturbance or N-deposition (Davis et al. 2000; Davis and Pelsor 2001; Seabloom et al. 2015). Interactions among such abiotic environmental changes and invasion mechanisms are likely, but rarely studied, resulting in a significant gap in our understanding of the drivers of invasion success (Bradley et al. 2010; Kardol et al. 2012).

A commonly cited mechanism behind invasion success that may interact with resource availability is belowground enemy release (Keane and Crawley 2002; Reinhart and Callaway 2006). Belowground enemy release refers to escape from the inhibitory effects of soil biota, such as root predation, parasitism, disease and competition for resources (Agrawal et al. 2005; Reinhart and Callaway 2006), which are assumed to be greater in a plant's native range due to higher abundances of co-evolved specialised enemies than in the introduced range, where soil biota are evolutionarily naïve of the invader. The benefits of belowground enemy release may also be magnified by increased nutrient availability. According to the growth rate

49 hypothesis, high resource environments, where the cost of replacing tissue is lower than

50 defending it, select for fast growing species (Coley et al. 1985; Stamp 2003), which are likely

51 to be regulated more heavily by enemies than slower growing, better defended, species

52 (Blumenthal 2006). Since invasive plant species tend to have more exploitative trait values

53 than co-occurring natives, such as higher relative growth rates (RGR) (Leishman et al. 2007,

54 2014; van Kleunen et al. 2010; Ordonez et al. 2010), they are well positioned to benefit from

55 the interaction of belowground enemy release with increased resource supply (Blumenthal

56 2006).

57 Such interactions are likely to be particularly important in grassland ecosystems, where

58 changes in nutrient availability are common due to intensification and invasion rates are

59 among the highest worldwide (Firn et al. 2011). In addition, grasses are the functional group

60 that generally show the most negative plant-soil feedbacks and are therefore most likely to

61 benefit from belowground enemy release (Kulmatiski et al. 2008). However, the invasive

62 success of different grass species, as measured by their abundance in their native versus their

63 introduced range, can vary (Firn et al. 2011). This suggests that grassland species responses

64 to plant-soil feedbacks and nutrient availability may be species-dependent. Here, we use a

65 native New Zealand grassland as a model system. These grasslands are valuable conservation

66 habitats (Mark and McLennan 2005; Rose and Frampton 2007) that are experiencing

67 invasions by a range of non-native species including several grass species, along with parallel

68 declines in native species abundance (Duncan et al. 2001; Rose et al. 2004). As the invasive

69 grasses in this system tend to have more exploitative traits and a higher RGR than the native

70 grass species (Craine and Lee 2003; Gross et al. 2013), and invasion appears to be facilitated

71 by increases in nutrient availability (Williams 1998; Scott 2000; Dickie et al. 2014), it

72 provides an ideal context within which to test how plant-soil feedbacks and nutrient

73 availability interact to influence invasive species growth, and whether these effects are

74 consistent across invasive species. In particular, we hypothesise that:

75     1. Belowground enemy release interacts with increased nutrient availability to promote

76        growth of three common invasive grass species, *Lolium perenne* L., *Anthoxanthum*

77        *odoratum* L. and *Agrostis capillaris* L., in grassland soil from their introduced range

78        (New Zealand) compared to their native range (United Kingdom).

79     2. Invasive grass species differ in the benefit they receive from the interaction of

80        belowground enemy release and nutrient availability.

81

82 **Materials and Methods**

83 FOCAL SPECIES

84 We used three perennial C3 grass species, *L. perenne*, *A. capillaris* and *A. odoratum,* that are

85 native to the UK and invasive in many parts of the world, including New Zealand (CABI

86 2017). These species were chosen as they are among the most widespread invasive grasses in

87 New Zealand (CABI 2017), yet they differ in their invasion success rates, in terms of their

88 relative abundances "home" and "away" (Firn et al. 2011) and so may vary in their responses

89 to belowground enemy release and nutrient addition. They were also introduced to New

90 Zealand at a similar time; *A. capillaris* in 1867, *A. odoratum* and *L. perenne* both in 1855

91 (New Zealand Plant Conservation Network 2016), which controls for differences in the

92 accumulation of belowground enemy pressure due to time since introduction (Diez et al.

93 2010). We used a common native perennial C3 New Zealand grass, *Poa cita,* that co-occurs

94 with the invaders in their introduced range (Gross et al. 2013). This served as a model native

95 species, which is not invasive anywhere, to which we could compare the responses of the

96  invaders. Seeds of all species were sourced from NZ populations by Speciality Seeds and

97  Home Creek Nursery, except *A. odoratum* which was supplied by B&T World Seeds.

98  SOIL COLLECTION

99  In April 2015, we collected soils from five indigenous montane grassland sites in New

100  Zealand (NZ) and five upland grassland sites in the United Kingdom (UK) (Table 1). British

101  colonisers of New Zealand introduced livestock and pasture grasses from the UK. It is

102  therefore likely that the invasive grass species used in our study originated from UK

103  populations and we therefore chose the UK as the source of our native range soil. Field sites

104  within each country were at least 20 km apart. Sites were suitable habitat for the focal species

105  (*A. capillaris*, *A. odoratum, L. perenne* and *P. cita*), not intensively managed and relatively

106  low fertility. At each site, soil cores (diameter = 6 cm, depth = 10 cm) were taken from 36

107  points spaced 10 m apart along six 60 m transects, covering an area of c. 5400 m$^2$ and

108  amounting to c. 10 L of soil per site. The trowel used to collect soil was sterilized between

109  sites using 30% bleach and rinsed in DI water to avoid any cross contamination of microbes.

110  Abundances of each focal species were also estimated within a 1 m$^2$ quadrat at each soil core

111  location. Focal species occurred at low mean abundance ($< 7\%$) at each site, representing the

112  early stages of invasion, and there were no significant differences in mean abundance

113  between the UK and NZ ranges. Fresh soil was sieved (4 mm) and homogenised within each

114  site, keeping sites separate to maintain independence (Reinhart and Rinella 2016). Soil was

115  transported on ice to Lancaster University (UK) where experiments were conducted and was

116  stored at 4$^0$C prior to use in the experiment. A subsample of c. 2 L of soil collected from each

117  site was then sterilized via gamma irradiation at 40 kGy (Synergy Health, UK).

118  EXPERIMENTAL DESIGN

119    To determine how different species responded to nutrient addition when grown with soil

120    biota from their native and introduced ranges we conducted a greenhouse experiment using a

121    randomised block design with five replicates. Treatments consisted of a full factorial cross of

122    soil origin (UK or NZ), sterilization (live or sterilized), nutrient addition (control and nutrient

123    addition) and four plant species (*A. capillaris, A. odoratum, L. perenne* or *P. cita*) grown in

124    monoculture, resulting in 160 pots. Live and sterilized soil was used to assess the effects of

125    soil biota from each range. This holistic approach allows the net effect of both beneficial,

126    such as arbuscular mycorrhizal fungi (AMF), and antagonistic soil biota to be assessed, and

127    thus gives a realistic picture of the impact of soil feedbacks on invasion success (Reinhart and

128    Callaway 2004; Gundale et al. 2014; Maron et al. 2014). Nutrient addition consisted of 30

129    mL 0.25 strength Hoagland's solution (Hoagland and Arnon 1950) per pot each week,

130    resulting in 22.4 mg N and 3.95 mg P being added over the study period.

131    GREENHOUSE CONDITIONS

132    Focal species were germinated in an autoclaved growing medium that consisted of sand and

133    peat (2:1 ratio by volume). This was done in the greenhouse under the same standardised

134    conditions that were used throughout the experiment: lighting regime: L: D 16h: 8h, Temp

135    $22^{o}$C: $16^{o}$C. Seeds were surface sterilized in 95% ethanol (1 min), then 6% sodium

136    hypochlorite (5 mins), then rinsed repeatedly with de-ionised water for 10 mins (Bartelt-

137    Ryser et al. 2005) in order to destroy any microbes that may have been adhering to the

138    surface of seeds prior to sowing. All equipment (e.g. pots) was sterilized in 30% bleach and

139    well rinsed with de-ionised water. Pots (1.5 L, diameter 15 cm) were filled with 1350 mL of

140    the same autoclaved growing medium in which the seeds were germinated (sand: peat mix).

141    This was then inoculated (i.e. gently mixed) with 150 mL (10 % of pot volume) of fresh

142    homogenised soil from either a UK or NZ site that was either gamma-irradiated (sterilized) or

143    live (unsterilized). This method tested differences in soil biota between similar habitats in the

144 native (UK) and introduced ranges (NZ), whilst minimising physical and chemical soil

145 differences. Final concentrations of KCl extractable N concentration ($NO_3^-$-N and $NH_4^+$-N)

146 and $NaCO_3$ extractable $PO_4^-$-P concentration (Olsen-P) in inoculated pots were determined

147 colorimetrically in a segmented flow stream using an AutoAnalyser (Seal-Analytical). Mean

148 concentrations of soil inorganic N were 3.3 ug N $g^{-1}$ higher in the growing medium

149 inoculated with UK soils ($10.6 \pm 0.6$ ug N $g^{-1}$) than that inoculated with NZ soils ($7.3 \pm 0.5$

150 ug N $g^{-1}$; $F = 44.2, p < 0.01$). This difference amounted to 4.4 mg N per pot, which was

151 relatively minor compared to the amount of N added in the nutrient addition treatment (22.4

152 mg N $pot^{-1}$) and it was the same across live and sterilized soils. Soil Olsen-P concentrations

153 and pH (soil: water, 1: 2.5) did not differ between UK and NZ soil. Mean concentrations of

154 soil inorganic N were 4.1 ug N $g^{-1}$ higher in sterilized soil ($11.0 \pm 0.5$ ug N $g^{-1}$) compared to

155 live soil ($6.9 \pm 0.4$ ug N $g^{-1}$; $F = 66.8, p < 0.01$), while Olsen-P concentrations were 0.7 ug P

156 $g^{-1}$ higher in sterilized soil ($1.4 \pm 0.1$ ug N $g^{-1}$) than live soil ($0.6 \pm 0.1$ ug N $g^{-1}$; $F = 17.9, p <$

157 $0.01$). These differences were the same across UK and NZ soils. Soil was left in pots for two

158 weeks to stabilise (Zuppinger-Dingley et al. 2011), then three seedlings of the same species

159 were transplanted into the pots on 7th May 2015 at the start of the experiment. Any seedlings

160 that died within the first week were replaced. Pots were watered daily with 60 mL of DI

161 water and re-adjusted to 80% water holding capacity of the growing medium twice each

162 week. Blocks were rotated every two weeks to minimise the effects of differences in

163 environmental conditions within the greenhouse. Plant biomass was harvested after 17 weeks

164 on 3rd September 2015. All soil was washed from roots and biomass was separated into

165 belowground and aboveground components and dried at 65 $^0$C for 48 hours before being

166 weighed to 0.0001g. Root mass fraction (RMF = belowground biomass/ total biomass) was

167 calculated in addition to biomass as it is an important plant trait that indicates the resource

168 investment into roots versus shoots. This provides insight into plant species growth strategies

169    and influences on plant growth due to above and belowground conditions. Soil inorganic N

170    and P concentrations were also measured at the end of the experiment. Soil inorganic N

171    concentrations were low and slightly higher in live soil ($0.11 \pm 0.03$ ug N g$^{-1}$) than sterilized

172    soil ($0.02 \pm 0.003$ ug N g$^{-1}$; *F = 9.56, p < 0.01*), whilst they did not differ in relation to

173    nutrient addition treatment ($F = 1.38$, $p = 0.24$). Soil Olsen-P concentrations were also low

174    and slightly higher in NZ soil ($0.38 \pm 0.03$ ug P g$^{-1}$) than UK soil ($0.27 \pm 0.02$ ug P g$^{-1}$; *F =*

175    *7.89, p < 0.01*), they also did not differ in relation to nutrient addition treatment ($F = 1.37$, p

176    = 0.24).

177    We determined the RGRs of each species as they provide a good indication of how

178    exploitative or conservative species are in their traits overall. This may be relevant for

179    interpreting differences in species responses to belowground enemy release and nutrient

180    additions. RGRs were determined by measuring the change in mean above and belowground

181    seedling biomass (M) between days 14 (t1) and 29 (t2) after germination (Pérez-

182    Harguindeguy et al. 2013). Twenty seedlings were harvested and dried (65 $^{0}$C for 48 hours) at

183    each time point. RGRs were calculated as:

184    $RGR = (\ln M_2 - \ln M_1) / (t_2 - t_1)$

185    STATISTICAL ANALYSIS

186    We split our analysis into two elements; one for each hypothesis. To test our first hypothesis,

187    we determined whether belowground enemy release and increases in nutrient availability

188    were interacting to influence individual species biomass responses (mean total biomass (g)

189    and mean root mass fraction). To do this, we conducted a three-way ANOVA with soil origin

190    (NZ or UK), sterilization (live or sterilized), nutrient addition (control and nutrient addition)

191    and all interactions as factors, on the biomass responses of each species independently. To

192    test our second hypothesis, we determined whether species differed to each other in their

193  responses to sterilization and nutrient addition depending on soil origin (NZ or UK). To do

194  this, we conducted a three-way ANOVA with species identity, sterilization, nutrient addition

195  and all interactions as factors, on the biomass responses in NZ and UK soil separately.

196  ANOVAs used type II sums of squares and therefore conformed to the principle of

197  marginality (Fox and Weisberg 2011), this was necessary as one replicate each of *A.*

198  *capillaris*, *A. odoratum* and *L. perenne* were lost due to contamination in seed supply,

199  resulting in a slightly unbalanced design. Tukey HSD post-hoc tests were used to assess pair-

200  wise significant differences ($p < 0.05$) between the levels of a factor, including any

201  interacting factors. Where significant interactions between factors were found in our three-

202  way ANOVA models, we also decomposed the analysis by separating the data into smaller

203  sections based on the groups of one of the significant factors. This allowed us to gain a

204  greater insight into which mechanisms were influencing biomass responses. Block did not

205  have a significant effect on the biomass responses of any individual species, nor on overall

206  biomass responses in NZ or UK soils and was therefore not included as a random effect.

207  Models that violated assumptions of normality or homoscedasticity received a $\log_{10}(y)$

208  transformation and all analyses were performed in R version 3.2.4 (R Core Team 2016).

209

210  **Results**

211  INTERACTION OF BELOWGROUND ENEMY RELEASE AND NUTRIENT ADDITION

212  When species were analysed independently (to answer hypothesis 1), their total biomasses

213  were all significantly higher when grown with either soil that originated from the UK; soil

214  that had been sterilized (regardless of origin) and when receiving nutrient addition (Table S1

215  and figs. 1 & S1-4). There were no significant interactions between soil origin (UK or NZ)

216 and sterilization treatment (sterilized and live) across any of the species (Table S1). The mean

217 total biomass of *L. perenne* only increased significantly in response to nutrient addition when

218 grown in soil originating from its introduced range (NZ), not its native range (UK), as

219 indicated by a significant interaction between soil origin and nutrient addition (*F = 4.6, p =*

220 *0.04,* Table S1, fig. S3a). However, when *L. perenne*'s total biomass was analysed in NZ soil

221 only, there was no interaction between sterilization treatment and nutrient addition (*F = 1.3,*

222 *p = 0.28*).

223 All species showed a higher RMF in sterilized soil than live soil (Table S1; figs S1-4), while

224 *A. capillaris* and *L. perenne* also both showed a higher RMF in NZ soil than UK soil (Table

225 S1; figs. S1 & S3). There were no interactions between any factors in the ANOVAs on RMF

226 for any species (Table S1).

227 INTERACTION OF SPECIES IDENTITY WITH NUTRIENT ADDITION

228 When species were analysed collectively (to answer hypothesis 2), differences in how they

229 responded to increased nutrient availability depended on the biogeographic origin of the soil

230 they were grown with (Table 2). In UK soil, all species responded similarly to nutrient

231 addition, as indicated by a lack of interactions between nutrient addition and other factors

232 (Table 2; fig. 1c & 1d). In contrast, in NZ soil there was a significant interaction between the

233 effects of sterilization and nutrient addition treatments on total biomass; with species

234 responding more strongly to nutrient addition in sterilized soil than live soil (*F = 5.6, p =*

235 *0.02*; Table 2). To gain further insight into this result, we decomposed the analysis by

236 sterilization treatment; thereby testing the effects of nutrient addition and species identity in

237 live and sterilized NZ soil separately (Table 3, fig. 1a & 1b). In live NZ soil, *L. perenne*

238 responded more strongly to increased nutrient availability than the other species in terms of

239 its total biomass (fig. 1a); as indicated by an interaction between species identity and nutrient

240    addition (*F = 3.5, p = 0.03;* Table 3). Tukey HSD post-hoc tests showed that while all

241    species except *A. capillaris* responded positively to nutrient addition in live NZ soil, *L.*

242    *perenne* responded most strongly (fig. 1a). It attained a significantly higher mean total

243    biomass than all other species in the nutrient addition treatment but not the control treatment

244    (fig. 1a). In sterilized NZ soil, however, species total biomass responded similarly to nutrient

245    addition, as indicated by the lack of an interaction between species identity and nutrient

246    addition (Table 3; fig 1b).

247    Differences in RMF between species depended on sterilization treatment in both soil origins,

248    as indicated by a significant interaction between species identity and sterilization treatment (*F*

249    *=3.6, P = 0.02* and *F = 4.0, P = 0.01*; NZ soil and UK soil respectively, Table 2, fig. 2). All

250    species except *L. perenne* showed a significantly lower RMF in live NZ soil than sterilized

251    NZ soil (Table 2, fig. 2a). Moreover, *L. perenne* maintained a higher RMF in live NZ soil

252    than both *A. capillaris* and *P. cita* (fig. 2a). The native grass *P. cita* showed the lowest RMF

253    in NZ soil (fig. 2a). In UK soil, all species showed similar RMFs except *A. capillaris,* which

254    exhibited a much lower RMF in live UK soil (fig. 2b).

255

256    **Discussion**

257    Belowground enemy release did not appear to be a strong factor influencing invasion success

258    in our study. All invasive species showed higher growth in soil from their native range (UK)

259    and the net effect of removing soil biota via sterilization was positive regardless of where

260    soils were from. Nevertheless, biogeographic differences in soil biota affected species

261    responses to nutrients in ways that have implications for their invasion success. In particular,

262    there was strong evidence to suggest that the presence of soil biota in the introduced range

263    (NZ) enabled *L. perenne* to respond more strongly to nutrients than all other species, as its

264  growth response to nutrients was stronger when grown in live NZ soil than other species

265  responses (fig. 1a). In contrast, all species responded similarly to nutrients when grown with

266  soil biota from the native range (UK) or in sterilized soil (figs. 1b – d). Unlike many invasive

267  grasses, including *A. capillaris* and *A. odoratum*, *L. perenne* generally shows a greater

268  abundance in its introduced range than its native range (Firn et al. 2011). Our findings

269  suggest that the mechanisms underlying these differences in species relative abundances

270  across their native and introduced ranges may relate to differences in soil biota and nutrient

271  acquisition, even in the absence of belowground enemy release.

272  There are two likely ways in which the presence of soil biota from the introduced range could

273  enhance *L. perenne's* acquisition of nutrients relative to other co-occurring species. Firstly,

274  beneficial soil organisms such as AMF could directly increase *L. perenne's* access to

275  nutrients more than they do other species. While most vascular plant species, including

276  grasses, are capable of forming mutualistic associations with AMF, they vary in the degree of

277  benefit they receive (Heijden et al. 1998; Klironomos 2003). Invasive plant species may be

278  more likely to form mutualistic associations with generalist AM fungi (Reinhart and

279  Callaway 2006; Moora et al. 2011), although research into this is still in its early stages

280  (Dickie et al. 2017). *L. perenne* can benefit substantially from associations with generalist

281  AM fungi, such as *Glomus spp.* (Cliquet et al. 1997; Faure et al. 1998; Torrecillas et al. 2014)

282  and may have developed more positive mycorrhizal associations in introduced soil than other

283  species. Secondly, competition for nutrients from the introduced soil biota may have had a

284  more negative effect on other species than on *L. perenne* (Niu et al. 2016; Zhu et al. 2016,

285  2017). Our study design did not allow us to separate mutualistic or antagonistic effects of soil

286  biota and therefore the exact mechanism remains uncertain.

287  In addition to soil biota effects, it is possible that *L. perenne* has some other characteristic that

288  allows it to perform differently to the other species. For example, *L. perenne* had the highest

289 RGR in our study (0.24), which suggests it may prefer high resource environments compared

290 to the other species. However, the other species also varied in their RGRs; *A. capillaris*

291 (0.21), *A. odoratum* (0.18) and *P. cita* (0.16), yet they showed no consistent differences in

292 their responses to nutrient addition in any soil. Perhaps more significantly, *L. perenne* showed

293 a higher RMF than both *A. capillaris* and *P. cita* in live soil from its introduced range (NZ).

294 Furthermore, it was the only species that did not show a reduced RMF in live soil compared

295 to sterilized soil from its introduced range (fig 2a). Maintaining a relatively high RMF could

296 enable it to take up additional nutrients more effectively by pre-empting supply (Craine et al.

297 2005), thus providing a clear competitive advantage. Interactions between invader root traits

298 and biogeographic variation in soil biota are therefore likely to be important for

299 understanding plant invasions. Belowground traits, such as nutrient acquisition strategy, can

300 influence plant-soil feedbacks (Bennett et al. 2017; Teste et al. 2017) and are increasingly

301 recognised as drivers of ecological processes (Bardgett et al. 2014). Our findings suggest that

302 they may also be important for understanding species invasions, particularly in the context of

303 increasing nutrient availability due to pervasive environmental change.

304 Whilst biogeographic differences in soil biota were important in controlling species responses

305 to nutrients in our study, we found no evidence for belowground enemy release. The role of

306 belowground enemy release in driving species invasions varies across species and localities

307 (Mitchell and Power 2003; Chun et al. 2010; Sun et al. 2014; Maron et al. 2014). Many of the

308 studies that found strong effects assessed invasive trees or forbs, and used North American

309 and European soils (e.g. Reinhart and Callaway 2004; Gundale et al. 2014; Maron et al.

310 2014). Fewer studies seem to have found evidence for belowground enemy release driving

311 grass species invasions. This is surprising, as grasses generally show more negative plant-soil

312 feedbacks than other functional groups, and are therefore most likely to realise the benefits of

313 enemy release (Kulmatiski et al. 2008). Some European pasture grasses appear to have more

314    positive associations with soil biota in Californian grasslands than native grasses, although

315    whether this stems from belowground enemy release remains unclear (Bennett and Strauss

316    2012). In contrast, the native grass species in our study, *P. cita*, responded in a similar way to

317    the invasive grasses, showing higher growth in UK soil and a similarly positive response to

318    sterilization in soils from either origin. Therefore the growth of native and invasive grasses

319    appears to be constrained to a similar extent by belowground enemies in New Zealand. Only

320    having one co-occurring native species in our study limits the implications of any invasive –

321    native comparisons, although *P. cita* is widespread and therefore ecologically relevant as a

322    comparison. *P. cita* responded as positively to nutrients in live NZ soil as *A. odoratum* and *A.*

323    *capillaris*, although much less so than *L. perenne*. This suggests that while increases in

324    nutrient additions appear to facilitate invasive grasses in the field in NZ (Scott 2000; King

325    and Wilson 2006; Dickie et al. 2014), this is likely to be species dependent. Other factors,

326    such as disturbance and priority effects, i.e. where the first species to arrive following a

327    disturbance ultimately dominates the community (Seabloom et al. 2003), or superior

328    competitive abilities (Sun et al. 2014; Broadbent et al. 2017), likely underlie the invasions of

329    other grass species, including *A. capillaris* and *A. odoratum*. In combination with findings

330    from previous studies, our results suggest that predicting which invasive plant species are

331    most likely to benefit from belowground enemy release will be difficult, due to large

332    variation within functional groups and across different habitats in the introduced range.

333    When species responses were analysed individually, all species in our study showed

334    increased growth following nutrient addition. However, for *L. perenne* a positive growth

335    response was only seen in soils from its introduced range (fig. S3a). This increase did not

336    differ between live and sterilized soil from the introduced range, suggesting that it was not

337    due to differences in soil biota. Instead, differences in nutrient availability between UK and

338    NZ soils may explain this result. This is supported by our analysis of soil chemistry before

339    the experiment started, which indicated that NZ soils had a slightly lower initial inorganic N

340    content than UK soils, even after dilution with 90% of the peat and sand medium was taken

341    into account. This was, however, a snapshot measurement of soil nutrient concentrations, and

342    by the end of the experiment there were no differences between NZ and UK soil inorganic N

343    concentrations. The role of soil biota in driving species responses to nutrients only becomes

344    clear when individual species responses are analysed relative to co-occurring species. This

345    highlights the importance of studying multiple co-occurring invasive species in order to

346    elucidate the species-specific variation in invasion mechanisms.

347    We used soil that had been conditioned by natural vegetation communities as opposed to

348    experimentally pre-conditioning soil (Kulmatiski et al. 2008). Some studies pre-condition soil

349    prior to starting the experiment by growing artificial plant communities in it, thereby

350    conditioning the soil biota community on those particular plant species. We were interested in

351    how invasive plant species responded to nutrient additions when grown with soil biota that

352    had been conditioned by natural plant communities that are vulnerable to invasion following

353    nutrient increases, compared to similar communities in their native range. Our findings

354    therefore reflect processes occurring at the very early stages of invasion, following

355    colonisation by invasive species (Theoharides and Dukes 2007). Soils conditioned by fast-

356    growing species have been shown to have higher nitrogen availability than soils conditioned

357    by slow growing species (Baxendale et al. 2014). This subsequently improved the

358    competitive ability of fast-growing species later grown in those soils (Baxendale et al. 2014).

359    This effect could theoretically lead to the facilitative interaction of novel soil biota and

360    nutrient addition on fast-growing invasive species, such as *L. perenne*, becoming prolonged

361    throughout later stages of invasion, even if the original source of nutrient addition ceases.

362    Whether this could account for the higher abundances of fast growing invasive species, such

363 as *L. perenne,* in their introduced ranges relative to their native ranges, has to the best of our

364 knowledge never been tested, but would make an interesting avenue for further research.

365 CONCLUSION

366 Even when the net effect of an invasive plant's associations with soil biota in its introduced

367 range are negative, the presence of these novel soil biota may still allow it to respond more

368 strongly to nutrient additions than its competitors, compared to soil biota from the native

369 range. This mechanism may contribute to the invasive success of some species, and suggests

370 that the range of plant-soil feedbacks associated with successful invasion is far wider than

371 that encompassed in the belowground enemy release hypothesis. We also found evidence that

372 belowground plant traits, such as RMF, may be important in driving responses, although

373 assessing whether this is a general trend or not would require testing across a wider range of

374 species than that tested here. Considering the globally widespread nature of anthropogenic

375 nutrient additions to ecosystems, the effects seen in our study could be contributing to a

376 global homogenisation of flora and the associated losses in native species diversity (Firn et al.

377 2011; Seabloom et al. 2015; Van Kleunen et al. 2015).

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**Tables**

**Table 1** List of field sites from where soil was collected in the U.K. and New Zealand, with

elevation (m) and location (WGS 1984/ Lat. Long.)

| Site | Country | Elevation (m) | Latitude | Longitude |
|------|---------|---------------|----------|-----------|
| Edale | UK | 507 | 53.374149 | -1.8304451 |
| Bradfield | UK | 306 | 53.443550 | -1.6111165 |
| Longshaw | UK | 334 | 53.315296 | -1.6070889 |
| Great Dunn Fell | UK | 671 | 54.670539 | -2.4440604 |
| Hartside | UK | 551 | 54.766721 | -2.5596763 |
| Clearwater | NZ | 655 | -43.59602024 | 171.01760960 |
| Lynton | NZ | 859 | -43.30431126 | 171.70230002 |
| Craigieburn | NZ | 818 | -43.14667393 | 171.73990218 |
| Turton | NZ | 943 | -43.35302069 | 171.36680554 |
| Tekapo | NZ | 1180 | -43.83077613 | 170.63581736 |

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642 **Table 2** Results of 3-way ANOVAs testing effects of species identity (SP), sterilization (ST),

643 nutrient addition (N) and their interactions on total biomass (g) and root mass fraction (RMF)

644 of all species in New Zealand (NZ) and U.K. soil origin treatments. All factors are fixed

645 effects

| | | Total biomass | | RMF | |
|---|---|---|---|---|---|
| | $df$ | $F$ | $P$ | $F$ | $P$ |
| *NZ soil* | | | | | |
| SP | 3 | 9.5 | <0.01 | 30.5 | <0.01 |
| ST | 1 | 56.3 | <0.01 | 77.5 | <0.01 |
| N | 1 | 116.7 | <0.01 | 1.3 | 0.26 |
| SP x ST | 3 | 1.5 | 0.21 | 3.6 | 0.02 |
| SP x N | 3 | 0.2 | 0.92 | 0.9 | 0.46 |
| ST x N | 1 | 5.6 | 0.02 | 0.7 | 0.4 |
| SP x ST x N | 3 | 1.6 | 0.20 | 0.6 | 0.64 |
| *UK soil* | | | | | |
| SP | 3 | 3.5 | 0.02 | 14.9 | <0.01 |
| ST | 1 | 23.7 | <0.01 | 22.9 | <0.01 |
| N | 1 | 15.9 | <0.01 | 0.4 | 0.51 |
| SP x ST | 3 | 1.2 | 0.33 | 4.0 | 0.01 |
| SP x N | 3 | 0.3 | 0.81 | 0.4 | 0.73 |
| ST x N | 1 | 0.1 | 0.81 | 0.1 | 0.73 |
| SP x ST x N | 3 | 0.4 | 0.73 | 0.4 | 0.75 |

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648    **Table 3** Results of 2-way ANOVAs testing effects of species identity (SP), nutrient addition

649    (N) and their interaction on total biomass of all species in live and sterilized New Zealand

650    (NZ) and U.K. soils. All factors are fixed effects

|  | | Total biomass | |
|---|---|---|---|
|  | *df* | *F* | *P* |
| *Live NZ soil* | | | |
| SP | 3 | 14.1 | <0.01 |
| N | 1 | 91.6 | <0.01 |
| SP x N | 3 | 3.5 | 0.03 |
| *Sterilized NZ soil* | | | |
| SP | 3 | 3.5 | 0.03 |
| N | 1 | 53.8 | <0.01 |
| SP x N | 3 | 0.2 | 0.87 |
| *Live UK soil* | | | |
| SP | 3 | 2.0 | 0.13 |
| N | 1 | 6.4 | 0.02 |
| SP x N | 3 | 0.2 | 0.90 |
| *Sterilized UK soil* | | | |
| SP | 3 | 2.9 | 0.05 |
| N | 1 | 10.9 | <0.01 |
| SP x N | 3 | 0.7 | 0.56 |

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654 **Figure legends**

655 **Fig. 1** Total biomass responses of all species when grown in different soil treatments: a) live

656 New Zealand (NZ), b) sterilized NZ, c) live United Kingdom (UK) and d) sterilized UK. Bar

657 and whisker points indicate mean +/- SE (N = 5). Means within each nutrient treatment with

658 the same letter are not significantly different (Tukey HSD, $p > 0.05$); * indicates differences

659 in species biomass across nutrient treatments (Tukey HSD; $p < 0.05$). Because species did not

660 respond differently to nutrient additions in panels b) – d), only the overall significant total

661 biomass response (Tukey HSD; $p < 0.05$) to nutrient addition is indicated (see Table 3 for all

662 F and p values)

663 **Fig. 2** Root mass fraction (RMF) responses of all species when grown in different soil

664 treatments: a) New Zealand and b) United Kingdom soil. Bar and whisker points indicate

665 mean +/- SE (N = 10). Means within each sterilization treatment with the same letter are not

666 significantly different (Tukey HSD, $p > 0.05$); * indicates differences in species' RMF across

667 sterilization treatments (Tukey HSD; $p < 0.05$)

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Fig.1

Fig. 2