# Positive plant-soil feedbacks of the invasive *Impatiens glandulifera* and their effects on above-ground microbial communities

Z PATTISON[1]*, H RUMBLE[1 †], R A TANNER[2ǂ], L JIN[3¥] and A C GANGE[1]

[1]*School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK*

[2]*CABI, Bakeham Lane, Egham, Surrey, TW20 9TY, UK*

[3]*School of Pastoral Agricultural Science & Technology, Lanzhou University, POB 61, Lanzhou 730020, Peoples Republic of China*.

*Present address: *Biological & Environmental Science, University of Stirling, Stirling FK9 4LA, UK*

[†]Present address: *Department of Geography University of Portsmouth, Buckingham Building, Lion Terrace, Portsmouth, Hampshire PO1 3HE*

[ǂ] Present address: European and Mediterranean Plant Protection Organisation, Paris, France

[¥]Present address: *Natural History Research Center, Shanghai Natural History Museum, Shanghai Science & Technology Museum, Shanghai 200127, Peoples Republic of China*

Author for correspondence: zarah.pattison@stir.ac.uk

Running title: *Impatiens* plant-soil feedbacks

25 Word count: 6225

26

27 ## Summary

28 *Impatiens glandulifera* is one of the most widespread invasive plant species in the UK.
29 Although aspects of its biology are known, there is little information about its association
30 with microbial communities both above- and below-ground. Furthermore, it is unknown
31 whether this species exhibits any form of plant-soil feedback (PSF), commonly seen in other
32 invasive weeds. We conducted a PSF experiment, in which plants of *I glandulifera* were
33 grown in soil that supported the species, and compared with those in a control soil from the
34 same locality. Soil nutrients were measured and the soil and foliar microbial communities
35 were assessed. *I. glandulifera* grew larger and faster in conditioned soil compared to the
36 control. Higher levels of phosphate were also found in conditioned soils. Arbuscular
37 mycorrhizal fungal (AMF) colonisation was lower in conditioned soils, suggesting that *I.*
38 *glandulifera* may rapidly alter AMF communities in invaded areas. PSFs had a significant
39 effect on the foliar endophyte community, with clear separation of species between
40 conditioned and control soils. These results show that *I. glandulifera* displays a positive PSF
41 and the PSF mechanism extends beyond the soil microbial community to affect foliar
42 endophytes. The observed increase in endophytes in plants grown in conditioned soil could
43 enhance resistance to herbivory, thus further accentuating the invasive properties of this
44 species.

45

46 **Key-words:** endophytes, non-native invasive species, multitrophic interactions, mycorrhizal
47 fungi, plant-soil biota interactions

## Introduction

There is increased evidence that certain plant species can selectively alter the soil microbial community, creating a plant–soil feedback (PSF) that can directly influence plant growth and fitness (Kulmatiski *et al*., 2008). The direction of the feedback, whether positive or negative, is dependent on the net effect of antagonistic and mutualistic soil microbes on plant growth and fitness. Positive PSF can result in improved performance of conspecifics, whereas negative PSF can result in soil conditions that decrease the performance of conspecifics and promote the co-existence of other species (Kulmatiski *et al*., 2011). An accumulation of pathogenic microbes in the soil can have negative effects on plant species that cultivate them (Reinhart & Callaway, 2006), but aid species turnover and succession which leads to greater biodiversity in plant communities (Callaway *et al*., 2004).

Invasive non-native plant species tend to be associated with less negative PSF compared to native plants (Klironomos, 2002). Changes to the soil microbial community specific to individual invasive species may occur through various mechanisms. Nutrient changes in the soil, mediated by leaf litter and associated microbial decomposers, can alter nutrient availability within a plant community. In addition, shifts in soil nutrient levels as a result of root exudation or soil microbes, coupled with the accumulation of local pathogens, such as soil bacteria, may benefit conspecifics, whilst having detrimental effects on native plant species (Ehrenfield, 2010).

71 One such invasive non-native species is *Impatiens glandulifera* Royle (Himalayan
72 balsam). *I. glandulifera* is an annual species native to high altitude meadows in the Western
73 Himalayas, first introduced into Europe as a garden ornamental in the early 19$^{th}$ century
74 (Beerling & Perrins, 1993). In the UK, *I. glandulifera* forms dense monocultures along
75 riverbanks and within damp woodlands, where each plant can produce up to 2,500 seeds that
76 are propelled from ripened seed-pods up to 7 m from the maternal plant. Synchronous
77 germination of the seed bank, coupled with fast growth, enables the population to gain an
78 early season advantage over native annual herbs (Beerling & Perrins, 1993). Negative
79 impacts for this species have been recorded on ecosystem services (Chittka & Schurkens,
80 2001), native vegetation (Hulme & Bremner, 2006) and associated invertebrate populations
81 (Tanner *et al*., 2013). However, little is known about its effects on microbial communities.
82
83 Arbuscular mycorrhizal (AMF) and endophytic fungi form a ubiquitous symbiosis
84 with the majority of plant species within native habitats, and these associations are generally
85 mutualistic (Hartley & Gange, 2009). *I. glandulifera* is known to be weakly dependent on
86 AMF, and may act to significantly deplete the mycelial network below established
87 monocultures (Tanner & Gange, 2013; Ruckli *et al.,* 2014). Initial studies suggest that this
88 can act to significantly reduce the fitness of native plants known to be associated to habitats
89 that *I. glandulifera* invades (Tanner & Gange, 2013). However, we are unaware of any
90 studies that have evaluated effects on the whole microbial community (both above- and
91 below-ground) as a result of the presence of *I. glandulifera*.
92
93 The primary benefits conferred to plant species differ between AMF and foliar
94 endophytes, where the former provides enhanced nutrient acquisition through a mycelial
95 network and the latter provides resistance to environmental stresses and natural enemy

96 pressure (Hartley & Gange, 2009). Both AMF and endophytic fungi require photosynthate
97 from the host plant, which may lead to indirect competition between them, expressed through
98 the host plant (Eschen *et al*., 2010). Thus, as *I. glandulifera* is able to manipulate soil
99 microbes, these changes could affect foliar endophytes. This in turn may enhance resistance
100 to insect herbivores (Gange *et al*., 2012) or plant pathogens, producing a novel mechanism
101 for invasiveness. Endophytes may enhance the competitive nature of invasive plants
102 (Aschehoug *et al.,* 2012), but whether PSFs affect the endophyte communities of invasive
103 plants is unknown.
104
105 The aim of this study was to determine whether *I. glandulifera* exhibits any form of
106 PSF. Our objectives were to investigate whether soil bacterial abundance, AMF and nutrient
107 availability differ in soils that have and have not supported growth of the plant. Additionally,
108 we explored whether a PSF can extend above ground, to affect foliar endophytic
109 communities.
110

## 111 Methods

112 The PSF experiment was based on a two-staged approach, which involved the conditioning of
113 soil using *I. glandulifera* (Phase 1) and using *I. glandulifera* as a measure of soil changes
114 (Phase 2), following Kulmatiski & Kardol (2008).
115
116 *Impatiens glandulifera* seeds were collected from a large population at
117 Harmondsworth Moor, Middlesex, UK: 51°29 ‘58.2N, 000° 29.02.3″E in 2010. Seeds were
118 stored at 4°C for 6 months in order to break time-dependent dormancy. For each
119 experimental phase, seeds were surface sterilised and germinated on moist filter paper within
120 sterile Petri dishes, which were placed in an incubator at 4°C for approximately 4 weeks.

121

122 *Phase 1 – conditioning the soil*

123 Forty pots, each 13 cm in diameter, were filled with 500 g of soil collected from
124 within the grounds of the CABI Campus in Egham, Surrey, UK (51°25′10.7″N, 000°34 ′
125 16.9″W). The area where the soil was collected had not been cultivated for over 30 years,
126 with vegetation dominated by *I. parviflora* DC., *Urtica dioica* L., and *Quercus robur* L., and
127 had not been previously invaded by *I. glandulifera*. The site was cleared of vegetation prior
128 to experimental set-up in order to create space to position the pots. Control soil was treated in
129 the same manner as conditioned soil.

130

131 Two germinated seeds were placed in 20 pots, 1cm below the surface of the soil. The
132 remaining 20 pots were filled with soil but no seeds were added (control soil). All pots were
133 sunk into the ground with the rim flush against the soil surface, in a randomised block design.
134 After 14 days the weaker seedling was removed in each sown pot. Plants were maintained for
135 a total of 8 weeks (to maturity) ensuring individuals did not flower (*I. glandulifera* is listed
136 under Schedule 9 of the Wildlife and Countryside Act, 1981). At this point, leaves from each
137 plant were randomly selected and the endophytic community was sampled (see below for full
138 methodology). Following this, plants were harvested, roots were removed from the soil and 5
139 randomly selected pots from each treatment were analysed for soil nutrients (see below for
140 full methodology). All soil, both conditioned and control, was then left to air dry for 2
141 weeks, and thereafter stored in a cool, dark room for 8 months until commencement of the
142 feedback experiment (Phase 2) the following season.

143

144 *Phase 2 – evaluating I. glandulifera performance and microbial interactions in*
145 *conditioned soil*

146 For the second phase of the experiment, 20 pots were filled with 500 g of each soil treatment
147 i.e. that conditioned by plants the previous year and the unconditioned (control) soil. Two
148 germinated seeds from the same locality as above were placed in each of the 40 pots, though
149 this time pots were placed in a Controlled Environment Facility (16 h light/8 h dark at 23 ±
150 1°C, 35% relative humidity) for five weeks before placing outside to ensure seedling
151 establishment. Seedlings were watered daily with 100ml of water and after 14 days the
152 weaker seedling was removed. After 5 weeks, the pots were transferred to the same site as
153 above and sunk into the soil in a randomised block design, 4 m x 4 m in size. Plants were
154 maintained outside for 7 weeks and were watered 5 times a week with approximately 250 ml
155 of water. Individual plant height and leaf number was measured every 7 days. As before,
156 plants were harvested before flowering and total fresh biomass was recorded. The plants were
157 subsequently dried and weighed.
158
159 *Soil nutrient analysis*
160 Financial and logistical (ensuring sufficient soil for phase 2) constraints meant that soil could
161 not be sampled from all pots in the two growth phase experiments. Analysis of soil nutrients
162 was performed at the end of Phase 1, using soil from five randomly selected pots for each
163 treatment. The soil analysis was repeated at the end of Phase 2, where 10 pots were
164 randomly selected from each treatment. Following the soil nutrient analysis, the same 10
165 replicate units were subsequently assessed for phospho-lipid fatty acids (PLFA's),
166 endophytes and AMF colonisation (see below for methods). Approximately 30 g of soil was
167 removed weighed, dried and reweighed to calculate water content of the soil. Both fresh and
168 dried soil was used for the analyses. The extractable nitrate ($NO_3$) and phosphate ($PO_4$)
169 content for each treatment was measured using a Skalar segmented flow analyser according

170 to manufacturer's instructions. Potassium (K) was analysed by flame spectrometry using a

171 Perkin Elmer atomic absorption spectrometer Analyst 800.

172

173 *Phospho-lipid fatty acid profiling*

174 PLFA analysis was conducted to assess soil microbial communities following Frostegård *et*

175 *al.* (1993). Briefly, 3.00g ±0.05 (fresh weight) of soil was used from each pot. Lipids were

176 extracted by Bligh/Dyer solvent and phase separation performed with chloroform as an

177 organic solvent. Silica acid columns were used to fraction lipid material into neutral

178 (NLFAs), glyco- and phospholipids (PLFAs). Lipid methanolysis of PLFA and NLFA

179 fractions to obtain fatty acid methyl esters (FAMEs) was conducted in 0.2M methanolic KOH

180 and methylnonadecanoate (C19:0) was added as an internal standard. FAMEs were identified

181 by chromatographic retention times and bacterial PLFAs verified with a standard bacterial

182 FAMEs mix (Sigma-Aldrich, St Louis, USA). Analysis was performed by a Hewlett Packard

183 (HP) 5890 gas chromatograph equipped with a flame ionization detector and a DB-5 capillary

184 column (30 mm x 0.25 mm i.d., film thickness 0.25 μm). The injection temperature was

185 250°C and the detector temperature regime started at 100°C, increasing at 20°C min$^{-1}$ before

186 being held at 160°C for 5 minutes. Temperature increased again at 3.5°C min$^{-1}$ to 280°C

187 where it was held for 3 minutes before finally increasing at 20°C min$^{-1}$ to 320°C. Injection

188 was splitless and helium was used as a carrier gas. FAMEs were identified on an HP 5970

189 mass spectrometer.

190

191 Fatty acid nomenclature followed Frostegård *et al*., (1993). The abundance of

192 individual PLFA's is expressed as equivalent responses to the internal standard, in μg g$^{-1}$ dry

193 weight of soil (modified from Hedrick *et al*., 2005). Microbial markers were used to

194 characterize the community. The PLFAs 18:2ω6,9 (Frostegård *et al*., 2011) and 20:1ω9

195 (Sakamoto *et al*., 2004) were used as indicators of fungi while C14:0i, C15:0i, C15:0ai,

196 C16:1i, C16:0i, C16:1ω7c, C16:0(10Me), C17:0i, C17:0ai, C17:0cy, C17:0(10Me),

197 C18:1ω9c, C18:0(10Me) and C19:0cy (Zelles, 1999) were used to characterize total soil

198 bacteria.

199

200 *Endophytic fungal community*

201 Towards the end of Phase 1 and 2, plants from each treatment (i.e. phase 1 plants, plants in

202 conditioned and control soil in phase 2) were evaluated for endophytic fungal communities.

203 Three asymptomatic mature leaves from each plant were removed and three 9mm$^2$ leaf pieces

204 were cut from each leaf and surface sterilised using the following procedure, modified from

205 Schulz *et al*., (1993). Fragments were subjected to a sequence of 30 immersions in 100%

206 ethanol, sterile water, 4.7% household bleach, 100% ethanol and finally by four rinses in

207 sterile water. Sterilised leaf pieces were placed on potato dextrose agar (PDA) with

208 antibiotics (60 mg l$^{-1}$ penicillin G and 80 mg l$^{-1}$ streptomycin sulphate to inhibit bacterial

209 contamination). Plates were maintained at 20$^{\circ}$C for 2 weeks, until fungal colonies were seen.

210 Emerging fungal colonies were transferred to potato carrot agar (PCA) plates by using a

211 sterile blade to remove approximately a 3 mm$^2$ piece of the developing fungal hyphae. Once

212 sporulation had occurred, slides were prepared and the cultures were identified by B.C.

213 Sutton. Previous work indicated that there was almost no difference in endophyte species

214 richness obtained by culturing and molecular methods within a range of annual plants

215 (Hodgson, 2010). Therefore this method was chosen, particularly as cultures were needed for

216 future manipulative experiments to be undertaken.

217

218 *AMF colonization*

219 Plants from Phase 2 were evaluated for AMF colonisation using the acidified ink staining
220 method of Vierheilig *et al.* (1998). Plants were removed from their pot and roots washed free
221 of soil. A 2-5 g sub-sample from each root system was immersed in a 10% potassium
222 hydroxide solution (10% w/v: 10g KOH in 100ml aqueous solution) and placed in a water
223 bath at 80°C for 25 minutes. Thereafter roots were rinsed with water and blotted dry. Roots
224 were placed in clean vials and covered with staining solution (84.4: 15: 0.6, dH2O: 1% HCI:
225 Quink blue pen ink) in the water bath for a further 15 minutes. Colonisation was measured
226 using the cross hair eye piece method of McGonigle *et al*. (1990).

227

228 *Statistical analysis*

229 All analyses were conducted using plants from Phase 2 as replicates. All data sets were
230 checked for normality and homogeneity of variances prior to analyses and all percentage data
231 were arc sine transformed. All data violating assumptions of homogeneity were transformed
232 with logarithmic or reciprocal transformations. Linear mixed effect models (LMM) using the
233 nlme package in R (Pinheiro *et al*., 2012) was used to assess plant height and total leaf
234 number, as a response to the interaction between treatment and time. Plant replicate was
235 assigned as a random effect. Exploratory analyses of the data revealed that the explanatory
236 variable 'time' was quadratic. The quadratic term was only retained within the model
237 alongside the associated main effect when significant. A one-way ANOVA was used to
238 examine soil treatment effects on fresh and dry biomass, as well as soil $NO_3$, $PO_4$ and K
239 content and percentage root length colonized (% RLC) by AMF. One-way ANOVA's were
240 used to compare total mass of PLFAs, mass of bacteria attributed PLFAs alone and mass of
241 fungal attributed PLFAs alone between invaded and native (control) soil. PLFA community
242 analysis (Principal Component Analysis; PCA) was performed in R (R Core Team, 2014) by

243 including concentrations of individual PLFA's. Principal components were then extracted
244 and interpretation based on factor loadings. LMM's and ANOVAs were performed in R.
245
246 Endophyte isolation frequency (IF) was calculated for each fungal species by dividing
247 the number of isolations (individual colonies) of a fungal species per plant by the total
248 number of isolations of all fungal species in that plant (Gange *et al*., 2007). Differences in
249 endophyte species richness and IF of fungal species between treatments were examined with
250 a one-way ANOVA, following transformation of percentage data. Treatments where a
251 particular fungus was absent were excluded from these analyses. All analyses were conducted
252 with the UNISTAT® statistical package. NMDS was performed to examine differences in the
253 species composition of foliar endophyte communities between treatments. The significance
254 of the overall separation and subsequent differences between treatments was quantified with
255 Analysis of Similarity (ANOSIM) using the Community Analysis Package (CAP5) (Pisces
256 Conservation, Lymington).
257
258 **Results**
259
260 *Vegetation growth*
261 Soil conditioning with *I. glandulifera* had a marked impact on subsequent vegetative growth.
262 There was a significant interaction between time and treatment for both height ($P < 0.01$, See
263 supplementary material Table 1a) and leaf number ($P < 0.01$, See supplementary material
264 Table 1b), highlighting the faster growth rate of plants grown in conditioned soil compared to
265 control (Fig. 1a and b). The changes in stature were also seen in plant biomass. Both total
266 fresh ($F_{1,34} = 63.4$, $P < 0.01$) and dry ($F_{1,34} = 50.6$, $P < 0.01$) biomass was almost three times
267 greater in conditioned soil compared with control soil (Fig. 1c).

268

269 *Fig 1 near here*

270

271

272 ### *Soil nutrient analysis*

273 Conditioned soil had a significantly greater amount of $PO_4$ than control soil at the end of
274 Phase 1 ($F_{2,12} = 20.0$, $P < 0.001$) and Phase 2 ($F_{1,18} = 8.5$, $P < 0.01$) (Fig. 2a). There was no
275 difference in K content between the soils after Phase 1 ($F_{2,12} = 1.9$, $P > 0.05$), but control soil
276 contained more K than conditioned soil after Phase 2 ($F_{1,18} = 16.1$, $P < 0.01$) (Fig. 2b). There
277 was no difference in $NO_3$ content between the soils after either Phase 1 ($F_{2,12} = 2.8$, $P > 0.05$)
278 or Phase 2 ($F_{1,18} = 3.0$, $P > 0.05$).

279

280

281 *Fig 2 near here*

282

283

284 ### *Microbial communities*

285 Mass of total fatty acids was higher in conditioned than in the control soils ($F_{1,18} = 6.70$, $P <$
286 $0.05$). Mass of bacterial fatty acids was higher in the conditioned soil than in control soil ($F_{1,}$
287 $_{18} = 4.70$, $P < 0.05$), but there were no differences in fungal fatty acids between the two soil
288 treatments ($F_{1,18} = 2.02$, $P > 0.05$) (Fig. 3). Two principal components (PC's) explained the
289 majority of the variance, with PC1 explaining 69% and PC2 explaining 21% (See
290 supplementary material Table 2). Moreover, there was a clear separation between PLFA
291 communities in control and conditioned soils, with conditioned soils less variable in

292 abundance of PLFA's than control soil (Fig 4). It is important to note, however, that there
293 was overlap between PLFA's found in each soil treatment.
294
295 *Fig 3 near here*
296
297 *Fig 4 near here*
298
299 AMF root colonization was observed in all plants. Roots of plants from conditioned
300 soil had mean colonisation levels of 22.8% ± 3.69, significantly less than the 44.6% ± 1.91 in
301 plants from control soil ($F_{1,18}$ =27.6, $P < 0.01$).
302
303 Plants at the end of Phase 1 contained an average of 3.1 ± 0.44 endophyte species per
304 individual. In addition, this figure did not differ from the equivalent (i.e. control soil) plants
305 in Phase 2, which contained 2.7 ± 0.39 fungal species. However, However, plants from
306 conditioned soil in Phase 2 contained significantly more fungi (4.2 ± 0.35) than their
307 respective controls ($F_{1,18}$ = 7.9, $P < 0.05$). In Phase 1, a total of 14 endophyte species were
308 isolated from all plants (data not shown), while in Phase 2, 11 species were isolated from *I.*
309 *glandulifera*. Only five species, *Alternaria alternata*, *Cladosporium cladosporioides*,
310 *Colletotrichum gloeosporioides*, *Co. dematium* and *Epicoccum nigrum* were found in plants
311 from both soil treatments in Phase 2. All of these species were found in Phase 1. *Tritirachium*
312 *dependens* and *Sordaria humana* were only found in plants from conditioned soil, while
313 *Colletotrichum acutatum* and *Fusarium culmorum* were found in control soil plants only.
314 Statistical analyses were possible for three endophyte species in Phase 2, because other
315 species were so rare that data sets contained high numbers of zero values. The isolation
316 frequency of *A. alternata* in conditioned soil plants (31.9%) was over twice that in plants

317 from control soil (13.1%) ($F_{1,18}$ = 5.9, $P < 0.05$). *C. cladosporioides* showed a similar
318 difference between conditioned (23.6%) and control (9.4%) soil plants ($F_{1,18}$ = 14.1, $P <$
319 0.01). *E. nigrum* too showed a similar trend (conditioned soil: 13.3%; control soil 9.4%), but
320 this was not significant ($F_{1,18}$ = 0.5 $P > 0.05$).
321
322 The NMDS ordination clearly separated the endophyte fungal assemblages of control
323 and conditioned soil treatments (ANOSIM $R$ = 0.198, $P < 0.001$) (Fig. 5). ANOSIM
324 demonstrated that differences were between control and conditioned soil-grown plants in
325 phase 2 ($R$ = 0.178, $P < 0.01$), and between plants in Phase 1 and conditioned soil plants in
326 Phase 2 (R = 0.255, $P < 0.01$). However, there was no difference in fungal communities in
327 plants grown in Phase 1 and those in control soil in Phase 2 (R = 0.011, $P > 0.05$), indicating
328 that the potential community of endophytes infecting plants was the same in each year.
329
330 *Fig 5 near here*
331
332
333 **Discussion**
334 Invasive forb species frequently exhibit positive plant-soil feedbacks, yet all previous studies
335 have focused on below-ground processes (Meisner *et al.*, 2014). We have shown that not only
336 does *I. glandulifera* exhibit a positive PSF, but that these effects extend to above-ground
337 microbial assemblages also. The finding that *I. glandulifera* manipulates below-ground
338 communities of AMF and bacteria and above-ground foliar endophyte communities could
339 provide important insights in our understanding of microbial community interactions,
340 especially in light of the recent release of a fungal biological control agent against this
341 species in the UK (Tanner *et al.,* 2015).

342

343 When grown in previously conditioned soil, *I. glandulifera* was taller, produced more leaves,
344 grew at a faster rate, and produced higher biomass, suggesting that it displayed a positive
345 PSF. Characteristics such as increased size are used as correlates of fitness in PSF
346 experiments, with positive PSFs demonstrated through an increase in biomass of individuals
347 grown in previously conditioned conspecific soil (Kulmatiski *et al*., 2008). High growth rate
348 of invasive plants can often be attributed to available N in the soil (Dassonville *et al*., 2008).
349 However $NO_3$ content did not differ between the soil treatments in this study, suggesting that
350 variation in growth response may instead be due to increased P via root exudation or
351 differences in the soil microbial community.

352

353 It has been well documented that AMF facilitate the acquisition of P to plants
354 (Richardson *et al*., 2009). However, AMF also require C from their hosts, which can lead to
355 negative effects on plant growth at high levels of colonisation (Gange & Ayres, 1999). Here,
356 AMF root colonisation in conditioned soil was half that of plants grown in control soil. What
357 is interesting is that higher AMF colonisation seen in the control soil did not result in greater
358 vegetative growth, indicating that this species may have a low threshold of AMF
359 colonisation, after which the mutualistic association declines. Symbiosis between plant and
360 AMF is optimum when increased P uptake leads to an increase in plant growth. Colonisation
361 above this optimum may result in AMF taking carbon from the plant, changing the
362 association from mutualistic to parasitic (Gange & Ayres, 1999).

363

364 The reduced colonisation by AMF in conditioned soil is similar to that seen by Ruckli
365 *et al* (2014), who found that *I. glandulifera* invasion reduced the AM colonisation of
366 sycamore (*Acer pseudoplatanus* L.) saplings. What is not known is whether the reduction in

367 AMF by *I. glandulifera* is a generic depletion of fungal species or the result of certain fungal
368 species failing to associate with the plant and so being eliminated from the soil community
369 (Tanner & Gange, 2013). Certainly, molecular analyses of the fungal species present in soil
370 would be most instructive, and this is the subject of our current research. Whatever, the
371 mechanism, it is clear that the reduction in AMF could have important consequences for
372 other plant species establishing post *I. glandulifera* removal. For example, Tanner & Gange
373 (2013) found that two native species (*Plantago lanceolata* L. and *Lotus corniculatus* L.) had
374 reduced mycorrhizal colonisation and fitness when grown in soil previously dominated by *I.*
375 *glandulifera*.
376
377 In contrast to mycorrhizas, *I. glandulifera* appeared to increase bacterial biomass,
378 with that in conditioned soil being almost twice that in the control soil. Invasive species have
379 been shown to alter soil communities, but effects can be variable. For example, increases in
380 bacterial biomass have been observed after invasions of *Amaranthus viridis* L. in Senegal
381 (Sanon et al., 2009) but a recent meta-analysis (Meisner *et al.*, 2014) suggests that, in most
382 cases, exotic species have little effect on soil bacterial biomass. In the field, bacterial biomass
383 manipulation by the invasive plant may additionally be influenced by the native species
384 present (Belnap & Phillips, 2001), further complicating interpretation of soil biota effects on
385 invasives. Thus, species and location specific responses are observed and with little data
386 available for this phenomenon in *I. glandulifera*, this paper provides a first step in analysing
387 specific responses for its invasive properties.
388
389 In addition, there is extensive evidence that invasive plants alter soil bacterial
390 community structure (Coats & Rumpho, 2014) and our results support this. Sanon et al.,
391 (2009) studied bacterial rRNA's in soils invaded by invasive *A. viridis* and found that certain

392 species were more prevalent within invaded soils than in others. Our PCA results reflect this,
393 with some of the community shared in both soils. Sanon et al., (2009) demonstrate that rRNA
394 analysis on soil biota is possible and can obtain high resolution data, so this could be a
395 credible next step in studies of *I. glandulifera* PSF, building on the findings of the current
396 study and give insights into direct and indirect effects of soil bacteria on plant growth.
397
398 Invasive plant research has generally focused on phytocentric parameters and more
399 recently, the effect on soil microbial communities. However, whether PSF effects extend to
400 foliar microbial communities has been ignored. Endophytic fungi in forbs can have profound
401 effects on plant fitness as well as possible protection against plant pathogens (Currie *et al*.,
402 2014) and insect herbivores (Gange *et al*., 2012). Conditioning of soil by *I. glandulifera* had a
403 significant effect on subsequent infection by fungal endophytes with *I. glandulifera* plants
404 grown in conditioned soil more susceptible to infection.
405
406 It should be noted that the endophyte community within plants in Phase 1 and in
407 plants grown in control soil in Phase 2 was almost identical. The main source of infection by
408 endophytes is likely to be spore rain from the air, causing many localised infections, but not
409 resulting in systemic growth (Yan *et al.*, 2015). Furthermore, infection of seedlings within
410 soils seems not to occur either (Currie *et al*., 2014), supporting the idea that foliar
411 colonization occurs exclusively by an aerial route. Moreover, litterfall did not occur in this
412 study, reducing the possibility of leaf endophytes producing spores that could enter the local
413 soil. Thus, we can discount inter-annual variation in endophyte spore rain as an explanation
414 for the differences observed between control and conditioned-soil plants. Instead, it would
415 appear that the PSF caused by *I. glandulifera* has a considerable influence on the foliar
416 endophytic community.

417

418 Of the most common endophyte species identified, both *A. alternata* and *C.*
419 *cladosporioides* occurred more frequently in plants grown in conditioned soil. These species
420 are known to be ubiquitous in nature, yet still showed a difference in infection levels between
421 *I. glandulifera* plants grown in close proximity. Strains of both species can be
422 entomopathogenic and occur as endophytes (Vega *et al*., 2008), but whether they were so in
423 this study is unknown. Endophyte species richness was greater in plants grown in conditioned
424 soil and there was a clear separation in endophyte fungal communities between conditioned
425 and control soil treatments. This indicates that *I. glandulifera* generates a PSF that can
426 influence fungal endophyte communities. Taken together, the observations that *I*
427 *glandulifera* appears to acquire more endophytes and higher levels of certain species as a
428 result of its conditioning of soil could have important consequences for biological control of
429 this weed. A plant with more endophytes may be better defended against natural enemies and
430 thus any biological control agent (see Tanner *et al.,* 2015). Indeed, Aschehoug *et al* (2012,
431 2014) have shown that *A. alternata* infection can have dramatic impacts on the highly
432 invasive *Centaurea stoebe* L., through increases in its competitive ability and allelopathic
433 potential.

434

435 Both soil nutrients and AMF are known to affect plant growth. However, their effects
436 on foliar endophyte communities are less well known. The size of a plant seems to have little
437 effect on the endophyte community within (Currie *et al*., 2014) and therefore the variation in
438 size between conditioned and unconditioned soil may not be the cause of differences in
439 endophyte communities seen here. Soil nitrogen and AM fungi have been shown to affect the
440 composition of endophyte species in the perennial forb, *Cirsium arvense* L. (Eschen *et al.*
441 2010). However, there was no difference in nitrate content between the two soil treatments in

442 this study. The influence on endophyte community composition in the current study may
443 therefore be due to the reduction in AMF inoculum potential in soil by this plant (Eschen *et*
444 *al*., 2010). This interaction has not previously been considered as a mechanism that might
445 promote invasiveness (Bennett, 2013) and deserves further investigation.
446

447 ### *Conclusion*

448 Our results show that *I. glandulifera* produces a positive PSF, manipulating both the
449 soil microbial and foliar endophyte community, as well as altering nutrient levels in the soil.
450 The effect on the foliar endophytes may be a secondary one, caused by changes in the
451 mycorrhizal levels and/or species in the soil. These findings may have profound implications
452 for understanding the invasive nature of weed species. Changes in the soil microbial
453 community, caused by a weed, may lead to changes in the foliar endophytes associated with
454 these plants. These changes may result in a 'perfect storm' whereby the weed is better
455 protected against predators and pathogens, making invasiveness more likely and biological
456 control more difficult. We conclude that future efforts at biological control and weed
457 management must take the plant-associated microbiome into account.
458

459 ## Acknowledgements

460 We are grateful to Neil Morley, Mark Levy, Jonquil Peck and Iñaki Valcarcel for their
461 technical assistance. We are grateful to Dr B.C. Sutton for the identification of endophyte
462 cultures. This research was funded in part by the Department for Environmental, Food and
463 Rural Affairs (Defra), the UK Environment Agency (EA) and the National Natural Science
464 Foundation of China (31270558).
465

466 ## References

467 ASCHEHOUG ET, CALLAWAY RM, NEWCOMBE G, THARAYIL N & SEN S (2014)
468 Fungal endophyte increases the allelopathic effects of an invasive forb. *Oecologia* **175**, 285-
469 291.
470 ASCHEHOUG ET, METLEN KL, CALLAWAY RM & NEWCOMBE G (2012) Fungal
471 endophytes directly increase the competitive effects of an invasive forb. *Ecology* **93**, 3-8.
472 BEERLING DJ & PERRINS JM (1993) *Impatiens glandulifera* Royle (*Impatiens roylei*
473 Walp.) *Journal of Ecology* **81**, 367-382.
474 BELNAP J & PHILLIPS SL (2001) Soil biota in an ungrazed grassland: Response to annual
475 grass (*Bromus tectorum*) invasion. *Ecological Applications* **11**, 1261-1275.
476 BENNETT AE (2013) Can plant–microbe–insect interactions enhance or inhibit the spread of
477 invasive species? *Functional Ecology* **27**, 661-671.
478 CALLAWWAY RM, THELEN GC, RODRIGUEZ A & HOLBEN WE (2004) Soil biota and
479 exotic plant invasion. *Nature* **427**, 731-733.
480 CHITTKA L & SCHURKENS S (2001) Successful invasion of a floral market - An exotic
481 Asian plant has moved in on Europe's river-banks by bribing pollinators. *Nature* **411***, 653.*
482 COATS VC & RUMPHO ME (2014) The rhizosphere microbiota of plant invaders: an
483 overview of recent advances in the microbiomics of invasive plants. *Frontiers in*
484 *Microbiology* **5**, article number 368, DOI: 10.3389/fmicb.2014.00368.
485 CURRIE AF, WEARN JA, HODGSON S, WENDT H, BROUGHTON SJ & JIN L (2014).
486 Foliar fungal endophytes in herbaceous plants: A marriage of convenience? In: *Advances in*
487 *Endophytic Research* (eds VC VERMA & AC GANGE) 61-81, Springer, New Delhi.
488 DASSONVILLE N, VANDERHOEVEN S, VANPARYS V, HAYEZ M, GRUBER W &
489 MEERTS P (2008) Impacts of alien invasive plants on soil nutrients are correlated with initial
490 site conditions in NW Europe. *Oecologia* **157**, 131-140.

491 ESCHEN R, HUNT S, MYKURA C, GANGE AC & SUTTON BC (2010) The foliar
492 endophytic fungal community composition in *Cirsium arvense* is affected by mycorrhizal
493 colonization and soil nutrient content. *Fungal Biology* **114**, 991-998.
494 FROSTEGÅRD Å, TUNLID A & BÅÅTH E (1993) Phospholipid fatty acid composition,
495 biomass, and activity of microbial communities from two soil types experimentally exposed
496 to different heavy metals. *Applied and Environmental Microbiology* **59**, 3605-3617.
497 FROSTEGÅRD Å, TUNLID A, & BÅÅTH E (2011) The use and misuse of PLFA
498 measurements in soils. *Soil Biology and Biochemistry* **43**, 1621-1625.
499 GANGE AC & AYRES RL (1999) On the relation between arbuscular mycorrhizal
500 colonization and plant 'benefit'. *Oikos* **87**, 615-621.
501 GANGE AC, DEY S, CURRIE AF & SUTTON BC (2007) Site- and species-specific
502 differences in endophyte occurrence in two herbaceous plants. *Journal of Ecology,* **95** 614-
503 622.
504 GANGE AC, ESCHEN R, WEARN JA, THAWER A & SUTTON BC (2012) Differential
505 effects of foliar endophytic fungi on insect herbivores attacking a herbaceous plant.
506 *Oecologia* **168**, 1023-1031.
507 HARTLEY SE & GANGE AC (2009) Impacts of plant symbiotic fungi on insect herbivores:
508 mutualism in a multitrophic context. *Annual Review of Entomology* **54**, 323-342.
509 HEDRICK B, PEACOCK A & WHITE DC (2005) Interpretation of fatty acid profiles of soil
510 microorganisms. In: *Monitoring and Assessing Soil Bioremediation.* (eds R. MARGESIN &
511 F SCHINNER), 251-259. Springer-Verlag Berlin .
512 HODGSON S (2010).. Foliar fungal endophyte dynamics in herbaceous hosts. Ph.D. thesis,
513 Royal Holloway University of London.

514 HULME PE & BREMNER ET (2006) Assessing the impact of *Impatiens glandulifera* on
515 riparian habitats: partitioning diversity components following species removal. *Journal of*
516 *Applied Ecology* **43**, 43-50.
517 KARDOL P, CORNIPS NJ, van KEMPEN MML, BAKX-SCHOTMAN TJM & van der
518 PUTTEN WH (2007) Microbe-mediated plant-soil feedback causes historical contingency
519 effects in plant community assembly. *Ecological Monographs* **77**, 147–162.
520 KLIRONOMOS JN (2002) Feedback with soil biota contributes to plant rarity and
521 invasiveness in communities. *Nature* **417**, 67-70.
522 KULMATISKI A & KARDOL P (2008) Getting plant-soil feedbacks out of the greenhouse:
523 experimental and conceptual approaches. In: *Progress in Botany* (ed U. LUTTGE) 449-472.
524 Springer-Verlag Berlin.
525 KULMATISKI A, BEARD KH, STEVENS JR & COBBOLD SM (2008) Plant-soil
526 feedbacks: a meta-analytical review. *Ecology Letters* **11**, 980–992.
527 KULMATISKI A, HEAVILIN J & BEARD KH (2011) Testing predictions of a three-species
528 plant-soil feedback model. *Journal of Ecology* **99**, 542-550.
529 KULMATISKI A, BAARD KH & HEAVILIN J (2012) Plant–soil feedbacks provide an
530 additional explanation for diversity–productivity relationships. *Proceedings of the Royal*
531 *Society, Biological Sciences* **279**, 3020–3026.
532 MEISNER A, HOL WHG, DE BOER W, KRUMINS JA, WARDLE DA & van der
533 PUTTEN WH (2014) Plant–soil feedbacks of exotic plant species across life forms: a meta-
534 analysis. *Biological Invasions* **16**, 2551-2561.
535 McGONIGLE TP, MILLERS MH, EVANS DG, FAIRCHILD GL & SWAN JA (1990) A
536 new method which gives an objective measure of colonization of roots by vesicular-
537 arbuscular mycorrhizal fungi. *New Phytologist* **115**,495-501.

538 R CORE TEAM (2014) R: A language and environment for statistical computing. R
539 Foundation for Statistical Computing, Vienna, Austria.
540 PINHEIRO J, BATES D, DEBROY S, SARKAR D & R CORE TEAM (2012) Nlme: Linear
541 and Nonlinear Mixed Effects Models. R package version 3.1-103.
542 REINHART KO & CALLLAWAY M (2006) Soil biota and invasive plants. *New*
543 *Phytologist* **170**, 445–457.
544 REINHAART KO, PACKER, A, van der PUTTEN WH & Clay K (2003) Plant–soil biota
545 interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology*
546 *Letters* **6**, 1046-1050.
547 RICHARDSON AE, BAREA J, McNIELL AM & PRIGENT-COMBARET C (2009)
548 Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by
549 microorganisms. *Plant and Soil* **321**, 305-339.
550 RUCKLI, R, RUSTERHOLZ H-P & BAUR B (2014) Invasion of an annual exotic plant into
551 deciduous forests suppresses arbuscular mycorrhiza symbiosis and reduces performance of
552 sycamore maple saplings. *Forest Ecology and Management* **318**, 285-293.
553 SAKAMOTO K, IIJIMA R & HIGUCHI R (2004) Use of specific phospholipid fatty acids
554 for identifying and quantifying the external hyphae of the arbuscular mycorrhizal fungus
555 *Gigaspora rosea. Soil Biology and Biochemistry* **36**, 1827-1834.
556 SANON A, BEGUIRISTAIN T, CÉBRON A, BERTHELIN J, NDOYE I, LEYVAL C,
557 SYLLA SN & DUPONNOIS R (2009) Changes in soil diversity and global activities
558 following invasions of the exotic invasive plant, *Amaranthus viridis* L., decrease the growth
559 of native sahelian Acacia species. *FEMS Microbiology Ecology* **70,** 118-131.
560 SANON A, BEGUIRISTAIN T, CÉBRON A, BERTHELIN J, SYLLA SN & DUPONNOIS
561 R (2012) Differences in nutrient availability and mycorrhizal infectivity in soils invaded by

562 an exotic plant negatively influence the development of indigenous *Acacia* species. *Journal*
563 *of Environmental Management* **95**, 275-279.
564 SCHULZ B, WANKE U, DRAEGER S & AUST H-J. 1993 Endophytes from herbaceous
565 plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research* **97**,
566 1447-1450.
567 SCHWEITZER JA; BAILEY JK, FICHER DG et al. (2008) Plant-soil microorganism
568 interactions: heritable relationship between plant genotype and associated soil
569 microorganisms. *Ecology* **89**, 773–781
570 TANNER RA, POLLARD K, VARIA, S, EVANS HC & ELLISON CA (2015) First release
571 of a fungal classical biocontrol agent against an invasive alien weed in Europe: biology of the
572 rust, *Puccinia komarovii* var. *glanduliferae*. *Plant Pathology*. **64**, 1130 – 1139.
573 TANNER RA & GANGE AC (2013) The impact of two non-native plant species on native
574 flora performance: potential implications for habitat restoration. *Plant Ecology* **214**, 423–432.
575 TANNER RA, VARIA S, ESCHEN R, WOOD S, MURPHY ST & GANGE AC (2013)
576 Impacts of an invasive non-native annual weed, *Impatiens glandulifera*, on above- and
577 below-ground invertebrate communities in the United Kingdom. *PLOS ONE* **8**, 1-13.
578 VEGA FE, POSADA F, AIME MC, PAVA-RIPOLL M, INFANTE F & REHNER SA
579 (2008) Entomopathogenic fungal endophytes. *Biological Control* **46**, 72-82.
580 VIERHEILIG H, COUGHLAN AP, WYSS U & PICHÉ Y (1998) Ink and vinegar, a simple
581 staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental*
582 *Microbiology* **64**, 5004-5007.
583 VOEGELSANG KM & BEVER JD (2009) Mycorrhizal densities decline in association with
584 nonnative plants and contribute to plant invasion. *Ecology* **90**, 399–407.

585 WEARN, JA, SUTTON BC, MORLEY NJ & GANGE AC (2012) Species and organ
586 specificity of fungal endophytes in herbaceous grassland plants. *Journal of Ecology* **100**,
587 1085-1092.
588 YAN J, BROUGHTON SJ, YANG SL & GANGE AC (2015) Do endophytic fungi grow
589 through their hosts systemically? *Fungal Ecology* **13**, 53-59.
590 ZELLES L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the
591 characterization of microbial communities in soil: A review. *Biology and Fertility of Soils* **29**,
592 111-129.
593
594 **Figure legends**
595
596 **Fig. 1.** The change in mean (a) height and (b) leaf number over time for *I. glandulifera*,
597 between conditioned (closed circles) and control (open circles) soil treatments. Total mean
598 plant biomass (c) of *I. glandulifera* between conditioned and control soil treatments. Closed
599 bars indicate fresh, open bars indicate dry biomass. Error bars represent SEM.

600

601 **Fig. 2.** Differences in mean (a) phosphate and (b) potassium content of dry soil at the end of
602 the Phase 2 experiment. Error bars represent SEM.
603
604 **Fig. 3.** (a) Total, (b) bacterial and (c) fungal PLFAs in μg $g^{-1}$ dry weight between the two soil
605 treatments. Error bars represent SEM. Stars (*) denote a significant difference at the $< 0.05$
606 level.

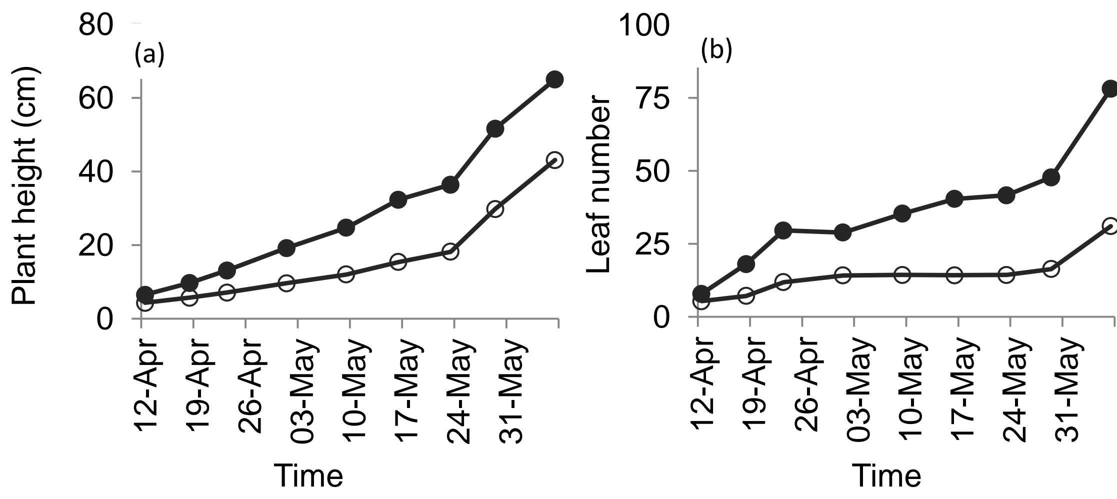
607

608 **Fig. 4.** Principal Components Analysis (PCA) ordination of soil phospholipid fatty acids
609 (PLFAs) from the two soil treatments carried out in phase two. Data points denoted by open
610 circles represent control treatments, open squares represent conditioned soil. Grey crosses,
611 presented for illustrative purposes, denote individual fatty acids (‘species’).

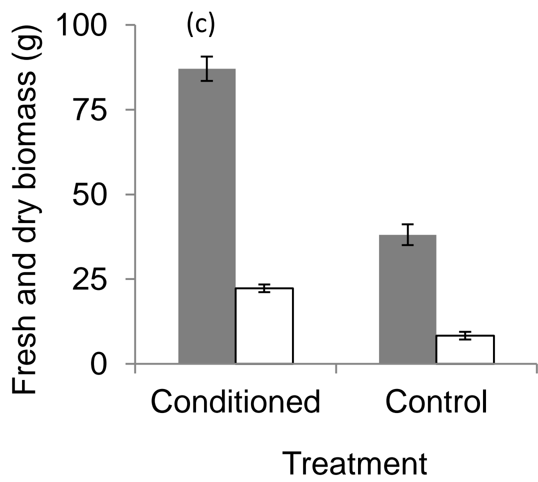
612

613 **Fig. 5.** Non-metric multidimensional scaling (NMDS) ordination of endophytic fungal
614 communities of *I. glandulifera* leaves from the three soil treatments. Data points denoted by
615 crosses represent plants at end of Phase 1, open circles represent plants in control soil in
616 Phase 2 and open triangles, plants grown in conditioned soil in Phase 2.

617 **Figures**




618

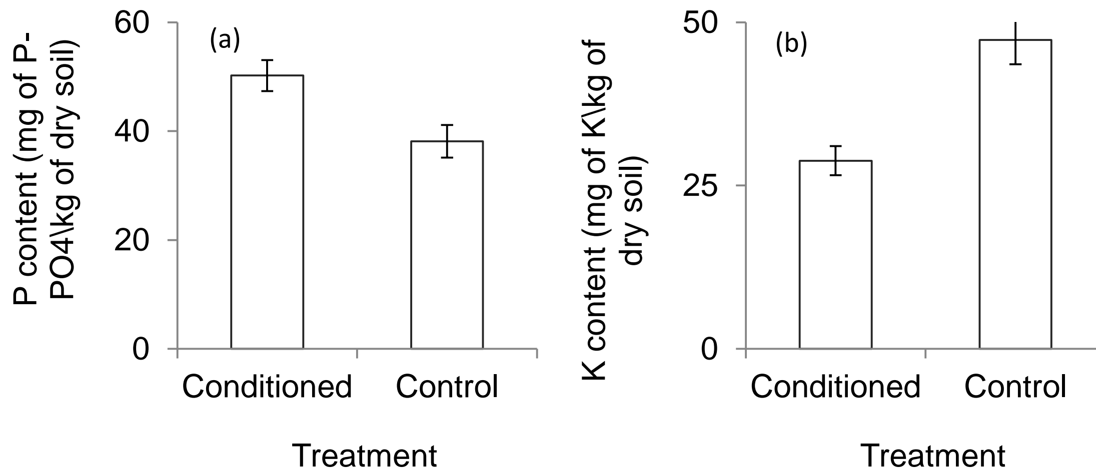



619

620 **Fig. 1.**

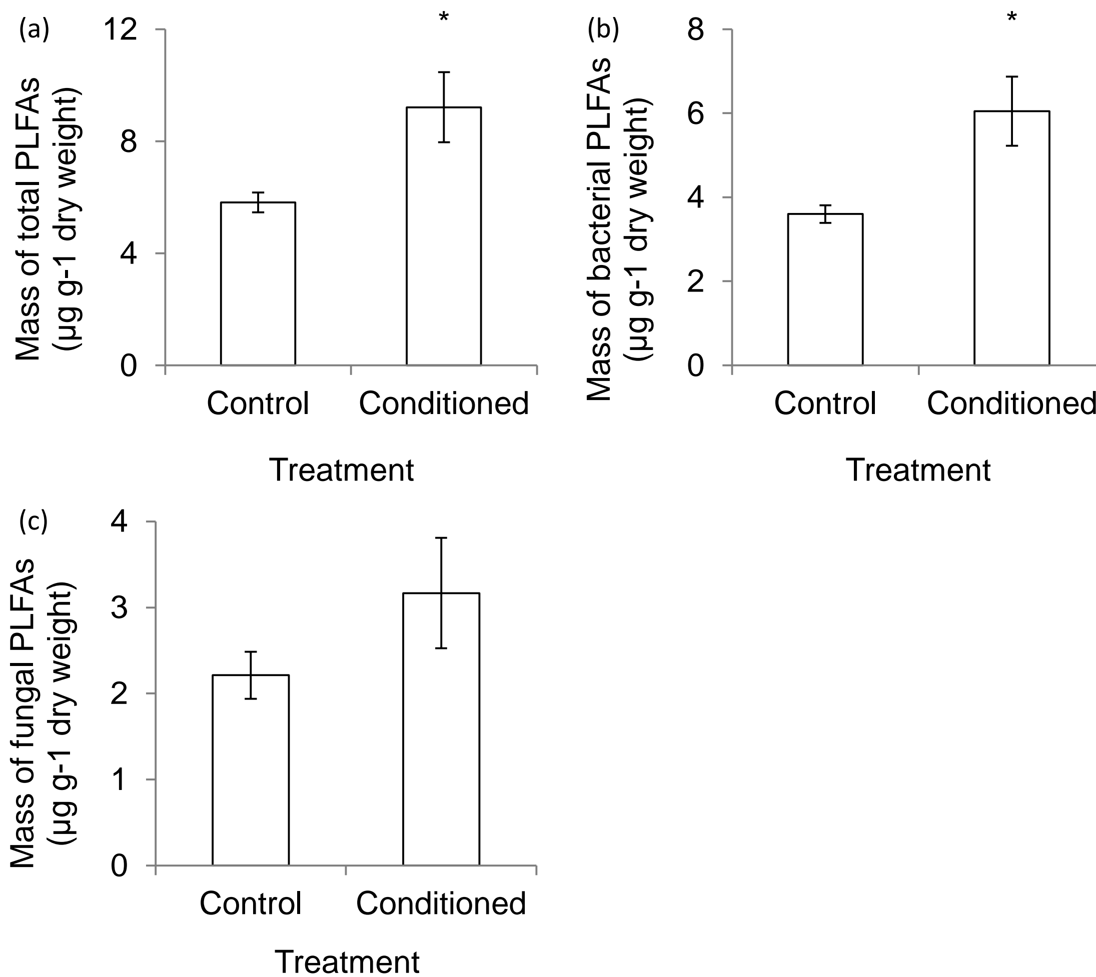
621

622




623 **Fig. 2.**

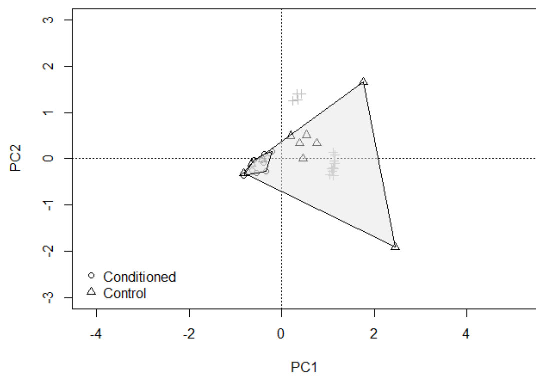
624




625

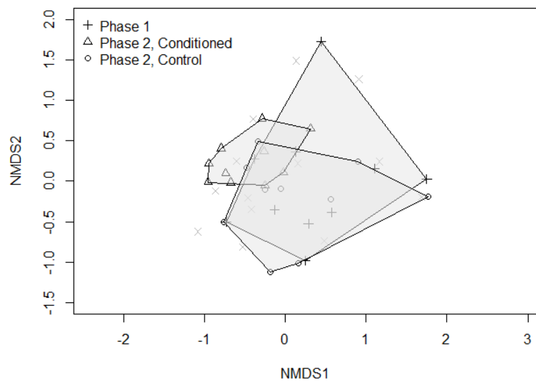
626 **Fig. 3.**

627




628

629 **Fig. 4.**




630

631 **Fig. 5.**