



Spatial mapping of root systems reveals diverse strategies of soil exploration and resource contest in grassland plants

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1 **Spatial mapping of root systems reveals diverse strategies of**
2 **soil exploration and resource contest in grassland plants**

3

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11

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18 MS and KZ designed the study; AL, MA, MS and KZ carried out the study; MS and JD
19 analysed the data; AL and MS wrote the first draft of the manuscript and all authors
20 contributed to revisions.

21 **Data accessibility**

22 Should the manuscript be accepted, the data supporting the results of this study will be
23 archived in Figshare, and the data DOI will be included at the end of the article.

24 **Abstract**

- 25 1. When foraging and competing for belowground resources, plants have to coordinate
26 the behaviour of thousands of root tips in a manner similar to that of eusocial
27 animal colonies. While well described in animals, we know little about the spatial
28 behaviour of plants, particularly at the level of individual roots.
- 29 2. Here, we employed statistical methods previously used to describe animal ranging
30 behaviour to examine root system overlap and the efficiency of root positioning in
31 eight grassland species grown in monocultures and mixtures along a gradient of
32 neighbour densities.
- 33 3. Species varied widely in their ability to distribute roots efficiently, with the majority
34 of species showing significant root aggregation at very fine spatial scales. Extensive
35 root system overlap was observed in species mixtures, indicating a lack of
36 territoriality at the level of whole root systems. However, with increasing density of
37 competitors, several species withdrew roots from the periphery of foraging ranges
38 and increased intraplant root aggregation in the remaining area, which may indicate
39 consolidation of foraging areas under competitive pressure.
- 40 4. Several species exhibited responses consistent with resource contest in species
41 mixtures where encounters with competitors' roots triggered increased root
42 aggregation at the expense of foraging efficiency. Such responses only occurred in
43 mixtures of species with comparable competitive abilities but were absent in
44 asymmetric species combinations.
- 45 5. *Synthesis*. Combining fine-scale measurement of plant root distributions with spatial
46 statistics yields new insights into plant behavioural strategies with significant
47 potential to impact resource foraging efficiency and productivity.

48

49 **Key words:** belowground interactions, competition, foraging efficiency, functional traits,
50 ideal free distribution, plant behaviour, plant-plant interactions, root spatial
51 distribution, root system overlap

52

53 **Introduction**

54 Plants produce extensive root systems often comprising kilometres of root length and
55 organised in complex branched structures. Due to a lack of easy tools to quantify and
56 characterise root spatial distributions, our knowledge of how roots are distributed in
57 soil at different spatial scales and how spatial patterns change in response to
58 competitive interactions is limited (Semchenko, John, & Hutchings, 2007b; Cahill et al.,
59 2010; Yang, Li, Xu, & Kong, 2018). On the other hand, root systems share qualities with
60 certain animals whose foraging and territorial behaviour has been extensively studied,
61 offering new ways of exploring and quantifying plant root behaviour (Novoplansky,
62 2009; McNickle & Brown, 2012; Ljubotina & Cahill, 2019).

63 Root systems may be comparable to eusocial central-place foragers such as bees
64 and ants, with root tips analogous to highly-related individuals serving the purpose of
65 foraging and delivering resources to a central “nest site”, i.e. in plants, the rooting point
66 connected to aboveground tissues. In common with animal colonies, root systems are
67 fundamentally constrained by the necessity to initiate all root growth from a single
68 rooting point (or a few rooting points in the case of clonal plants). Branching patterns
69 further constrain the spatial positioning of higher order branches (Robinson, Hodge, &
70 Fitter, 2003). In animal systems, a parallel to such constraints may be the use of
71 established paths or tunnels in ant and termite foraging that lead individuals to the most
72 profitable foraging areas (Traniello, 1989; Almeida et al., 2018). Beyond the constraints
73 of shared spatial origin, plant roots are expected to follow the ideal free distribution in
74 the absence of competitors, minimising intraplant competition and maximising resource
75 uptake efficiency (McNickle & Brown, 2014). Root system modelling indicates that
76 nutrient uptake efficiency is strongly influenced by root system topology and root

77 branching angles (Fitter, Stickland, Harvey, & Wilson, 1991; Ge, Rubio, & Lynch, 2000;
78 Lynch & Brown, 2001; Rubio, 2001; Dunbabin, Rengel, & Diggle, 2004). However,
79 empirical measurements of spatial distributions at the level of individual roots are
80 scarce (Caldwell, Manwaring, & Durham, 1991, 1996). Furthermore, while knowledge
81 about easily measured root morphological traits, and their links with community- and
82 ecosystem level processes, is rapidly accumulating (Bardgett, Mommer, & deVries, 2014;
83 Roumet et al., 2016; Kramer-Walter et al., 2016), it is unknown how these traits relate to
84 the efficiency of soil exploration at the level of individual roots. Revealing these
85 relationships could significantly improve our mechanistic understanding of fundamental
86 plant strategies.

87 Spatial behaviour is strongly influenced by interactions with neighbouring
88 individuals. Animals typically have an area that is regularly traversed in search of
89 resources (food and/or mates), known as the home range, which may be shared with
90 other groups or individuals (Adams, 2001). Depending on the costs of conflict and the
91 benefits of acquiring resources, different strategies are optimal, and different patterns of
92 home range overlap emerge (Sih & Mateo, 2001; Morrell & Kokko, 2005; Börger, Dalziel,
93 & Fryxell, 2008). When the cost of aggressive contest is very high and population
94 densities relatively low, the strategy of avoiding contest and seeking empty space is
95 most successful, leading to largely segregated home ranges (Stamps & Krishnan, 2001;
96 Morrell & Kokko, 2005). In plants, this scenario matches most closely to arid and semi-
97 arid ecosystems or communities with low densities of individuals where root system
98 segregation has indeed been recorded (Brisson & Reynolds, 1994; Schenk, Callaway, &
99 Mahall, 1999). When the costs of aggression are very low, the boldest and most
100 aggressive strategy is predicted to prevail, as the cost of sharing space is too low to deter
101 individuals from revisiting the same sites (Carpenter, 1987; Morrell & Kokko, 2005).

102 This scenario fits observations from mesic grasslands and forests where root systems
103 tend to overlap extensively (Fitter, 1987; Mamolos, Elisseou, & Veresoglou, 1995; Lang,
104 Dolynska, Finkeldey, & Polle, 2010; Mommer et al., 2010; Laclau et al., 2013, McNickle &
105 Brown, 2014). Furthermore, the degree of asymmetry among individuals in their
106 capacity to inflict damage (e.g. due to difference in size or resource uptake efficiency) is
107 expected to affect the outcome of interactions, leading to escalation or stalemate
108 between individuals of similar ability but unidirectional attack and escape when
109 individuals have asymmetric abilities (e.g. interspecific competition; Stamps & Krishnan,
110 2001; McNickle & Brown, 2012). The applicability of this theory to plants remains to be
111 tested.

112 Both theoretical predictions as well as empirical observations of plant root
113 systems have primarily focused on estimating total root production and root system
114 overlap at large spatial scales (Brisson & Reynolds, 1994; reviewed in Schenk et al.,
115 1999; Gersani, Brown, O'Brien, Maina, & Abramsky, 2001; Maina, Brown, & Gersani,
116 2002; Holzapfel & Alpert, 2003; O'Brien, Brown, & Moll 2007; Semchenko, Hutchings, &
117 John, 2007a; Cahill et al., 2010; McNickle & Brown, 2014). Spatial positioning of
118 individual roots has rarely been measured (Mahall & Callaway, 1991, 1992; Caldwell et
119 al., 1991, 1996; Semchenko et al., 2007b) but could significantly improve our
120 understanding of resource foraging efficiency in plants and provide a new perspective
121 on plant behavioural strategies such as resource contest and conflict avoidance. In this
122 study, we revisited the technique of freezing intact soil and mapping root spatial
123 locations (Caldwell et al., 1991, 1996) and combined it with georeferencing software
124 and statistical methods used to describe animal foraging and home range behaviour.
125 Using these tools, we explored root spatial distributions in a range of temperate
126 grassland species that were subjected to varying densities of conspecific and

127 heterospecific competitors. We expected that roots belonging to the same individual
128 would be aggregated at small spatial scales, indicating low efficiency of soil exploration,
129 due to the intrinsic constraints of central-place foraging, but the degree of root
130 aggregation would vary among species as a function of architecture (Fitter et al., 1991;
131 Dunbabin et al., 2004; Ge et al., 2000; Lynch & Brown, 2001; Rubio, 2001) and root
132 system size, with larger root systems less efficient at space exploration (Berntson, 1994;
133 Pagès, 2011). At larger spatial scales, we expected root systems to be more flexible and
134 therefore more likely to occupy space evenly (Gersani, Abramsky & Falik, 1998;
135 McNickle & Brown, 2014; Yang et al., 2018).

136 We also expected root spatial distribution to vary as a function of species
137 competitive ability. It has been predicted that combinations of individuals with
138 contrasting competitive abilities should produce lower root biomass compared with
139 mixtures comprising similar competitors (e.g. conspecifics), where escalating
140 overproliferation is likely (McNickle & Brown, 2012). However, no predictions have
141 been made regarding the efficiency of root placement. We suggest that escalating
142 resource contest among species with similar competitive abilities (reflected in the
143 similar biomass of competitors) may be manifested at fine spatial scales as aggregation
144 of competitors' roots and hence low efficiency of soil exploration. On the other hand,
145 interactions between species with contrasting competitive abilities may increase
146 intraplant root aggregation in competitively inferior species as a means of defending
147 their limited home ranges. Root positioning was recorded at two soil depths, to assess
148 the consistency of patterns along the soil profile, and at a range of neighbour densities to
149 explore how interactions changed with increasing competitive pressure. Lastly, we
150 related root spatial distribution and home range patterns to root morphological traits in
151 order to integrate root spatial behaviour with traits commonly used to describe plant

152 belowground strategies. We predicted that species with root traits reflecting a
153 competitive strategy, such as high specific root length and low tissue density (Wang,
154 Stieglitz, Zhou, & Cahill, 2010; Ravenek et al., 2016; Semchenko, Lepik, Abakumova, &
155 Zobel, 2018), would engage in resource contest by aggregating roots in the vicinity of
156 competitors. On the other hand, species with traits reflecting alternative resource
157 acquisition strategies, such as association with mycorrhizal fungi (e.g. reduced root
158 branching and large root diameter; Semchenko et al., 2018; Bergmann et al., 2020), were
159 predicted to exhibit efficient root placement and avoid neighbours' roots.

160

161 **Materials and methods**

162 *Experimental design and measurements*

163 The seeds of eight plant species (*Antennaria dioica* (L.) Gaertn., *Carlina vulgaris* L.,
164 *Filipendula vulgaris* Moench., *Galium verum* L., *Lotus corniculatus* L., *Pilosella officinarum*
165 Vaill., *Pimpinella saxifraga* L., *Sesleria caerulea* (L.) Ard.) were collected in 2008 from a
166 semi-natural, calcareous (alvar) grassland in Estonia (58°38'31"N, 23°30'55"E). The site
167 is characterised by high species richness and rendzic leptosol soil type, with an average
168 of 18 cm of humus layer over limestone shingle parent material (Pärtel, Kalamees, Zobel,
169 & Rosen, 1999; Nettan, Thetloff, Lepik, Semchenko, & Zobel, 2019). The seeds were air-
170 dried and stored at 4°C. The following spring, seeds were germinated on moist sand, and
171 similar-sized seedlings were transplanted into 3.5 l pots (17.1 cm diameter, 15 cm deep)
172 filled with a mixture of commercial potting compost (pH 6, water-soluble N 100 mg/l, P
173 80 mg/l, K 400 mg/l), sand and limestone powder. The germination of seeds from
174 different species was timed such that seedlings were available for transplantation at the
175 same time. The soil mixture was prepared to match the pH and N content of the soil at

176 the seed collection site as closely as possible. To provide plants with natural soil biota,
177 soil from the site of seed collection was also added to the mixture. Each species was
178 grown surrounded by either conspecific or heterospecific neighbours (species
179 monoculture and mixture treatments, respectively). Distributing experimental
180 replicates along a continuous gradient of environmental treatment with little or no
181 replication per treatment level can provide a more powerful approach for identifying
182 responses to environmental variation than examining fewer replicated levels of an
183 environmental treatment (Kreyling et al., 2018). Therefore, rather than employing the
184 frequently used approach of growing plants alone and at an arbitrary neighbour density,
185 we exposed plants to a gradient of seven neighbour densities (no neighbours, 1, 2, 3, 4, 6
186 or 8 neighbours) to obtain robust estimates of root spatial patterns and their responses
187 to neighbour density. The focal plant was planted at the centre of each pot and
188 neighbouring individuals were planted in a circle, equidistant from each other and at 5.7
189 cm distance from the focal plant (corresponding to 2/3 of the pot radius). Each focal
190 species was grown in the mixture treatment with a species that it frequently encounters
191 as its nearest neighbour in the field (Semchenko, Abakumova, Lepik, & Zobel, 2013).
192 Each focal species × neighbour density (1-8) combination was replicated twice and focal
193 plants grown in the absence of neighbours were replicated four times (224 pots in total;
194 212 measured at harvest due to seedling mortality).

195 Pots were placed randomly in a common garden at the end of May 2009 and re-
196 randomised twice during the experiment. Plants received natural precipitation and
197 additional watering during dry spells and were harvested after 14 weeks of growth.
198 Aboveground biomass was removed and dried at 70°C for 48 h and weighed. The roots
199 were left intact in the soil, which was then frozen at -18°C. The frozen pots were sliced
200 horizontally at depths of 5 cm and 10 cm below the soil surface. Roots were mapped at

201 two depths to determine if patterns of variation in root spatial distributions were
202 similar across different depths. The surfaces of defrosted soil slices were gently cleared
203 with water, to expose roots that had been sliced through, and scanned (Epson perfection
204 V700 PHOTO, Long Beach, CA, USA). The locations of all root intersections visible within
205 the central area of the scanned image, corresponding to 2/3 of the pot radius (Figs. 1
206 and S1 in Supporting Information), were recorded using ArcGIS software (version 10.0,
207 ESRI, Aylesbury, UK). The species examined in the mixture treatment had different root
208 colours, allowing focal and neighbouring plant roots to be visually distinguished; in the
209 species monoculture treatment, focal and neighbouring plant roots were not
210 distinguished. Hence, analysis of spatial patterns in monocultures was performed across
211 all plants in the pot while analysis of mixture data was performed for focal individuals
212 and neighbours separately.

213 To relate root spatial patterns to root morphology and architecture, we used root
214 trait data presented in Semchenko et al., 2018. These data were collected from the same
215 experiment, following the scanning of the soil slices by carefully washing roots out from
216 the top soil slices (0-5 cm depth) and separating the roots of focal and neighbouring
217 plants. From each focal plant and its neighbours, two representative root axes were
218 selected randomly for root morphological analysis. Roots were scanned (Epson
219 perfection V700 PHOTO, Long Beach, CA, USA), dried at 70°C for 48 h along with all of
220 the remaining roots and weighed. Root diameter, root length, root volume, and the
221 number of root tips were calculated using the program WinRhizo 2008a (Regent
222 Instruments Inc., Quebec, Canada). Using these measurements, root tissue density (dry
223 root mass/root volume), specific root length (root length/dry root mass), and root
224 branching frequency (number of root tips/root length) were calculated.

225

226 ***Estimation of root spatial patterns***

227 *Intraspecific root spatial distribution*

228 The degree of root spatial aggregation or segregation was calculated for each pot and
229 soil depth using the L function, which is derived by comparing Ripley's K function to the
230 pattern expected under complete spatial randomness (Ripley, 1976; Rowlingson &
231 Diggle, 2017). Ripley's K function ($K(t)$) is defined as the expected number of additional
232 points within a distance t of an arbitrary point within the study area, divided by the
233 overall density of points and corrected for edge effects. The L function was calculated as:
234 $L(t) = \sqrt{K(t)/\pi} - t$. The value of the function is zero if points are distributed
235 randomly in space (suggesting no response to the presence of neighbouring roots),
236 negative if points are overdispersed (indicative of root segregation and high efficiency of
237 space exploration), and positive if points are spatially aggregated (indicative of low
238 efficiency of soil exploration; Fig. 1). The L-function was calculated across all plants for
239 species monocultures and at the level of individual focal plants (i.e. reflecting intra-plant
240 spatial patterns) and neighbours for species mixtures. In this analysis, points
241 represented the locations of root intersections. Since the density of roots from a single
242 individual declines with distance from the rooting point and pots contained multiple
243 individuals, root density varied strongly across the pot, violating the assumption of
244 homogeneity that underpins the standard Ripley's K calculation (i.e. that the mean
245 intensity of the point pattern is constant across the observation area). To account for
246 variation in rooting densities across the pot, we used a generalisation of the L function
247 for inhomogeneous point patterns, which estimates the local intensity of the point
248 pattern using "leave one out" kernel smoothing (standard deviation of the Gaussian
249 kernel estimated based on the size of the observation area), with "translation" edge
250 correction, (function *Linhom* from R package *spatstat*, Baddeley, Rubak, & Turner, 2015).

251 To determine the scale of root architectural constraints and selective pressures on the
252 uptake efficiency of resources with different mobility, root spatial aggregation (L-
253 function) was calculated at two scales: $t = 2$ mm and $t = 1$ cm (Fig. S1), corresponding
254 respectively to the spatial scales of root depletion zones for less mobile (e.g. phosphate)
255 and more mobile (e.g. nitrate) ions (de Kroon, Mommer, & Nishiwaki, 2003).

256

257 *Interspecific root spatial distribution*

258 To assess how the roots of two species in mixtures were positioned in relation to each
259 other, bivariate L functions (L_{bv}) were calculated at two spatial scales (2 mm and 1 cm),
260 where the bivariate K function is the expected number of roots belonging to species 1
261 within a distance t of an arbitrary root belonging to species 2, divided by the overall root
262 density of species 1 and corrected for edge effects. To account for variable root densities
263 across the observation area, the inhomogeneous version of the bivariate L function was
264 calculated as described above (function *Ldot.inhom* in R package *spatstat*). L_{bv} is zero if
265 the roots of the two species are distributed randomly in relation to each other, negative
266 if roots are segregated, and positive if roots of one species aggregate with roots of the
267 other species (Fig. 1).

268

269 *Home range size and overlap*

270 Three metrics were calculated to describe the spatial extent of whole root systems and
271 overlap between the root systems of focal and neighbouring plants (Fig. 1). Minimum
272 convex polygon (MCP) area was used to describe the home range, i.e. the total area
273 encompassed by roots. The core foraging area was defined as the 50% kernel area based
274 on the bivariate normal kernel method (Worton, 1989), where a two-dimensional
275 probability density function describes the probability of finding roots in a given area

276 within a home range. Home range overlap in species mixtures was calculated as the total
277 kernel volume overlap (expressed as a proportion, with the maximum of 1 in case of
278 complete overlap). Ranges were calculated using package *adehabitatHR* in R (Calenge,
279 2006).

280

281 ***Statistical analysis***

282 Linear mixed models were used to estimate the effects of focal species (or species pair
283 identity in analyses of species mixtures; fixed factor, eight levels), neighbour density
284 (fixed factor, continuous variable), soil depth (fixed factor, two levels) and their
285 interactions on root spatial patterns. Pot identity was included as a random factor in all
286 models (as each pot was measured at two soil depths). The following spatial
287 characteristics were examined as response variables: a) L function in species
288 monocultures or species mixtures (focal plants and neighbours separately); and in
289 species mixtures b) bivariate L function (L_{bv}); c) MCP range of the focal plant; d) core
290 foraging area of the focal plant; and e) kernel volume overlap between focal and
291 neighbour plants. For L functions, separate models were performed for each spatial
292 scale (2 mm and 1 cm). Models were performed using package *lme4* in R (Bates,
293 Mächler, Bolker, & Walker, 2015).

294 To illustrate the results of linear mixed models, linear models were performed for
295 each species, treatment (species monoculture or mixture) and soil depth combination
296 with neighbour density as a continuous predictor and one of the spatial characteristics
297 described above as a response variable. In the absence of significant interactions
298 between species identity and neighbour density, predicted values at mean neighbour
299 density (four neighbours) were used to illustrate interspecific differences in spatial

300 patterns; otherwise, the estimated slope of the relationship between a spatial
301 characteristic and neighbour density was used to illustrate the effect of neighbour
302 density on each focal species.

303 We used data on plant shoot and root biomass, root tissue density, root branching
304 frequency, root diameter and specific root length from Semchenko et al. (2018) to
305 examine relationships between root spatial patterns and the size and morphological
306 traits of focal plants, as well as trait differences between focal and neighbouring plants,
307 which may reflect differences in competitive abilities or resource acquisition strategies.
308 For each focal species trait, predicted values at the mean neighbour density (four
309 neighbours) were extracted from a linear model with the trait as a response variable
310 and neighbour density as a continuous predictor. In addition, absolute differences
311 between focal and neighbour trait values were calculated for each pot in species
312 mixtures, and predicted absolute trait differences at the mean neighbour density were
313 extracted from linear models as described above. For shoot and root biomass, the
314 absolute difference between the focal plant mass and the total mass of all neighbours in
315 a pot was calculated.

316 In species monocultures, Pearson's correlations were examined between predicted
317 trait values and L function estimates. In the species mixtures, correlations were
318 examined between predicted trait values of the focal plant and range size (MCP and
319 core), and focal plant L function estimates. Lastly, correlations between absolute trait
320 differences (between the two species in the mixture) and home range overlap, and
321 bivariate L function (L_{bv}), were examined.

322 All data analyses were performed in R 3.6.3 (R Core Team, 2020).

323

324 **Results**

325 *Root spatial patterns in species monocultures*

326 Patterns of root spatial distribution varied significantly as a function of species identity,
327 soil depth and neighbour density and were dependent on the spatial scale of
328 observations (Table 1). At the finest (2 mm) spatial scale, roots were generally
329 significantly aggregated at the mean neighbour density (four neighbours) but varied
330 widely between species, and the monoculture of *Lotus corniculatus* exhibited random
331 spatial distribution (Fig. 2a). At the scale of 1 cm, roots were overall less aggregated
332 than at the 2 mm scale (Fig. 2b). Spatial aggregation patterns were consistent across soil
333 depths except for *Sesleria caerulea*, which showed significantly less root aggregation at
334 10 cm than 5 cm depth (depth by species interaction in Table 1, Fig. 2a). Root
335 aggregation generally declined with increasing neighbour density at the scale of 2mm
336 Fig. 3a, Fig. S2a), but no significant effect of neighbour density was detected at the scale
337 of 1 cm (Table 1).

338

339 *Root spatial patterns, range size and overlap in species mixtures*

340 Spatial aggregation of roots in species mixtures varied strongly between species pairs,
341 with *S. caerulea* showing most aggregated patterns and *L. corniculatus* nearly random
342 root distribution at mean neighbour density (Table 1, Fig. 2c-d). Contrary to species
343 monocultures, focal plants in species mixtures exhibited increased root aggregation with
344 neighbour density at 2mm spatial scale (Table 1, Figs. 3b and S2).

345 Intraspecific aggregation in neighbouring plants also varied significantly between
346 species mixtures (Table 1, Fig. 2e-f). Small-scale root aggregation in *S. caerulea*, which
347 was used as the neighbouring species in five species mixtures, differed significantly
348 between mixtures, with aggregation being significantly lower when grown in mixture

349 with *Carlina vulgaris* and *Pimpinella saxifraga* than when grown with the other three
350 focal species (Fig. 2e). Neighbours' roots became less aggregated with soil depth and
351 increasing neighbour density (Figs. 2e and 3c), though these effects varied among
352 species, particularly at the 1cm spatial scale (Table 1, Figs. 2f and S2).

353 Analysis of bivariate spatial patterns revealed that the roots of several species
354 were strongly aggregated in relation to each other at the 2 mm spatial scale (Fig. 2g). At
355 mean neighbour density, strong aggregation was observed in four species mixtures,
356 while three species combinations showed weaker aggregation or nearly random
357 distribution (Fig. 2g). Aggregation slightly declined with neighbour density (Table 1, Fig.
358 3d). At the larger spatial scale, interspecific root aggregation was reduced and roots in
359 the mixture of *F. vulgaris* and *S. caerulea* became significantly segregated (Fig. 2h).
360 Aggregation patterns were consistent across soil depths (Fig. 2g-h).

361 Focal plant core areas did not significantly shrink with increasing neighbour
362 density, while MCP ranges declined in *Antennaria dioica* and *S. caerulea* but not other
363 species (significant species by density interaction for MCP range in Table 2, Fig. 4a-b and
364 S3). These two focal species experienced a slight decline in home range overlap with
365 increasing neighbour density, while the other focal species showed increased overlap
366 (Fig. 4c and S3). Core area size and the extent of home range overlap generally increased
367 with soil depth (Table 2, Fig. S3).

368

369 *Relationship between root spatial patterns and plant traits*

370 In species monocultures, root spatial aggregation was not significantly related to the
371 total density of roots in soil but was significantly negatively correlated with root
372 diameter at both spatial scales (Fig. 5a, Table S1). In species mixtures, focal plant roots
373 were less aggregated at 2 mm spatial scale and occupied a larger range if the focal plant

374 attained high shoot and root biomass and high root density (Table S1, Fig. 5b-d).
375 Interspecific root aggregation (based on bivariate L-function) was negatively correlated
376 with the absolute difference in shoot mass between focal and neighbour plants (Table
377 S1, Fig. 5e). Range volume overlap was positively correlated with the absolute difference
378 between biomass and root density of focal and neighbour plants (Table S1, Fig. 5f).

379

380 **Discussion**

381 In this study, we obtained fine-scale measurements of root spatial distributions in a
382 range of grassland species and employed analytical approaches used in animal
383 behavioural studies to describe the foraging efficiency and home range behaviour of
384 plant roots. We found that plant species varied widely in their ability to secure a large
385 belowground home range and position their roots efficiently in space, representing new
386 axes of variation in plant strategies that can have important consequences for species
387 competitive ability, co-existence and efficiency of nutrient uptake. We also demonstrate
388 that roots belonging to different species often aggregated in each other's vicinity at very
389 fine spatial scales, which may represent resource contest at the expense of foraging
390 efficiency. In agreement with theoretical models of animal behaviour (Stamps &
391 Krishnan, 2001; Morrell & Kokko, 2005), such contests were more prevalent in
392 interactions between species of similar size while species pairs with asymmetric sizes
393 showed reduced confrontation. These findings suggest that complex evolutionary
394 models of behaviour are applicable to plants and should be considered as mechanisms
395 that can contribute to the effects of biodiversity on resource use efficiency and
396 productivity.

397 This study used a pot experiment to demonstrate a new approach to studying
398 plant root behaviour. However, the technique is amenable to other contexts and could
399 be combined with additional measurements to further explore the complexity of the
400 phenomenon. First, the species used in this study originate from calcareous grasslands
401 on thin soil and are characterised by slow growth and frequent experience of root
402 growth restriction in stony substrate. As such, they were well suited for a pot
403 experiment, where pot walls may restrict root growth. Pot walls may nonetheless have
404 affected the maximum extent of root systems, particularly for plants grown in the
405 absence of competitors. As a result, the response of focal root systems to neighbour
406 density may have been underestimated. Root morphology may also be affected by
407 interactions with pot walls (Semchenko et al., 2007a, Semchenko, Zobel, Heinemeyer, &
408 Hutchings, 2008). We therefore restricted our observations to the central area of the pot
409 and left areas adjacent to the pot walls as buffer zones. However, it is also conceivable
410 that roots that were not in direct contact with the pot walls were also affected. Such
411 effects might be particularly problematic for many species of larger size and those that
412 occupy more productive soils. It may therefore be critical to record root spatial
413 positioning under natural, field conditions. Second, in this study, all roots were included
414 in the assessment of spatial patterns. However, roots are likely to vary widely in their
415 physiological activity and may serve the function of nutrient uptake or transport. More
416 detailed knowledge of the costs and benefits of root positioning could be obtained if root
417 positioning were recorded in a time series, preferably over several growth seasons, and
418 combined with a technique enabling the estimation of physiological activity, e.g.
419 enzymatic activity (Razavi, Zarebanadkouki, Blagodatskaya, & Kuzyakov, 2016).

420

421 *Root spatial patterns in species monocultures*

422 The roots of most species were aggregated at fine spatial scales, likely reflecting
423 fundamental constraints on root architecture. Aggregation declined with increasing
424 spatial scale suggesting that plant root systems are less developmentally constrained
425 and able to occupy space more evenly at larger spatial scales. Wide variation in the
426 levels of aggregation was observed between species. Contrary to modelling studies
427 predicting that large root systems should be less efficient in root spatial positioning
428 (Berntson, 1994; Pagès, 2011), variation observed in this study could not be explained
429 by total root density. We found that species exhibiting lower levels of root aggregation
430 were characterised by larger root diameters. Large root diameter may reflect high root
431 construction costs as well as strong dependence on mycorrhizal associations, as a
432 thicker cortex layer offers space for fungal colonisation (Cortois, Schroder-Georgi,
433 Weigelt, van der Putten, & De Deyn, 2016; Kong et al., 2019; Bergmann et al., 2020).
434 Therefore, it may be that species with more costly roots experience stronger selective
435 pressure to position their roots efficiently in soil, even if such roots primarily play the
436 role of transport and nutrient exchange with fungal partners. However, this relationship
437 is only correlative and based on eight species, and hence requires further exploration
438 across a wider range of species and environmental conditions.

439 We found that root spatial aggregation in species monocultures (as well as
440 interspecific aggregation in species mixtures) tended to decline with increasing
441 neighbour density. This indicates that having more individuals per unit soil surface area
442 could be an important driver of efficient soil exploration, and ecological processes that
443 reduce plant mortality may significantly modify plant community functioning and
444 resource use efficiency. For example, niche complementarity or dilution of natural
445 enemy pressure in more diverse plant communities could support higher densities of
446 individuals (Peters, 2003; Marquard et al., 2009), which in turn could improve nutrient

447 foraging and transport efficiency, with potential positive effects on community
448 productivity.

449

450 *Root spatial patterns and range overlap in species mixtures*

451 We found that several species mixtures exhibited high levels of interspecific aggregation
452 at very fine spatial scales. Positioning roots very close to a competitor's roots may
453 reduce resource uptake efficiency but may intensify competition and thus represent a
454 contest of resources. Theoretical models of behaviour predict that interactions between
455 players with symmetric competitive abilities are likely to lead to escalated fights while
456 asymmetric interactions lead to reduced contest (Stamps & Krishnan, 2001;
457 Novoplansky, 2009). In line with these predictions, we found that interspecific root
458 aggregation was particularly pronounced in mixtures of species with similar biomass,
459 indicating similar competitive abilities (Keddy, Nielsen, Weiher, & Lawson, 2002; Wang
460 et al., 2010; Semchenko et al., 2018). In mixtures composed of species with very
461 different sizes, the home range of the larger competitor encompassed most of the range
462 of the inferior competitor. However, low levels of interspecific root aggregation were
463 observed despite the clear opportunity for contest. This observation is consistent with
464 an evolutionary model of root biomass production where in combinations of
465 competitors with contrasting competitive abilities it becomes obsolete for the superior
466 competitor to "harass" the weaker neighbour by producing extra roots, and the weaker
467 competitor curtails its root production in the face of a contest that could not be won
468 (McNickle & Brown, 2012).

469 Species mixtures differed significantly in how the roots of different species were
470 positioned in relation to each other but also how roots belonging to the same species

471 (intraspecific aggregation) were affected by the presence of other species. Aggregation
472 within individual focal root systems tended to increase with the increasing density of
473 heterospecific competitors. Such a response to increasing competitive pressure may
474 represent a strategy of consolidating occupied space and preventing further intrusion by
475 heterospecific roots, even if it comes at the cost of increased intraplant competition. It
476 could be achieved by switching from shallow rooting angles (most effective for avoiding
477 intraplant competition) to deeper angles that reduce root system overlap between
478 neighbours (Ge et al., 2000; Rubio, 2001). In line with this interpretation, we found that
479 home ranges declined in some species, while core area size remained stable, with
480 increasing neighbour density, suggesting that plants held on to their core foraging areas
481 but ceded space at the limits of their ranges with increasing competitive pressure.

482 Besides the density of competitors, the species identity of competitors also
483 significantly modified root placement. In our study, we created species mixtures by
484 coupling focal plants with neighbours that they encountered most frequently in the field.
485 This resulted in using one of the dominant grass species as the neighbouring species in
486 five mixtures. The root placement of this species was highly responsive to the identity of
487 the focal species. The grass showed highly aggregated root placement when combined
488 with three species but intraspecific root aggregation was much lower in other two
489 mixtures (levels similar to those observed in monoculture conditions). These
490 observations indicate that the efficiency of root placement differs significantly between
491 species but is also highly plastic within species in response to the density and identity of
492 competitors.

493 *Conclusions*

494 Fine-scale root spatial mapping can be combined with different statistical approaches to
495 gain new insights into plant belowground strategies and test the applicability to plants
496 of animal-based behavioural theories. The majority of game-theoretic models of plant
497 behaviour to date have focussed on predicting root biomass production over large
498 spatial scales. Here we go beyond assessing root biomass production and highlight the
499 importance of considering the spatial distribution of individual roots. Our study
500 uncovered a diverse range of ways plants deploy their roots in space. New modelling
501 approaches are now needed to explore which spatial strategies are evolutionarily stable
502 in the presence of competitors with different characteristics and why. We found that the
503 efficiency of root placement depended on the density and species identity of interacting
504 plants as well as their relative size. Therefore, community processes that determine
505 plant mortality and species richness and composition may play an important role in
506 regulating plant behavioural responses and associated efficiency of soil exploitation.
507 Finally, there were no clear links between spatial patterns and commonly measured root
508 morphological traits, indicating that root placement strategies may represent a distinct
509 trait axis that can significantly expand our understanding of plant belowground
510 strategies.

511

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696 **TABLES AND FIGURES**

697

698 **Table 1.** Results of linear mixed models examining the effects of species or species pair
 699 identity, neighbour density and soil depth on root spatial distribution (L-function) in
 700 species monocultures, intraspecific root distribution of focal (focal L-function) and
 701 neighbouring plants (neigh L-function) and interspecific root association in species
 702 mixtures (bivariate L-function). Rooting patterns were examined at two spatial scales (2
 703 mm and 1 cm). F-values and their significance are shown. \$ $P < 0.1$; * $P < 0.05$; ** $P <$
 704 0.01 ; *** $P < 0.001$. df – degrees of freedom. Residual degrees of freedom are based on
 705 Kenward-Roger approximations.

	df	Monoculture L-function		Mixture focal L-function		Mixture neigh L-function		Bivariate L-function	
		2mm	1cm	2mm	1cm	2mm	1cm	2mm	1cm
Species (S)	7	15.43 ***	4.50 ***	22.30 ***	6.29 ***	28.55 ***	5.54 ***	30.32 ***	3.21 **
Neighbour density (N)	1	12.23 ***	0.18	4.35 *	2.29	4.23 *	0.37	4.28 *	0.01
Depth (D)	1	0.11	1.82	2.30	0.65	5.30 *	1.17	3.50 \$	0.02
S:N	7	1.35	1.95 \$	1.02	0.76	0.56	1.72	0.44	0.43
S:D	7	3.07 **	3.24 **	1.25	1.90 \$	0.22	3.03 **	1.73	0.67
N:D	1	0.04	0.04	<0.01	0.01	<0.01	0.10	<0.01	<0.01
S:N:D	7	0.36	1.53	0.97	0.55	1.51	2.22 *	0.60	0.90
Res df		106		100		71		71	

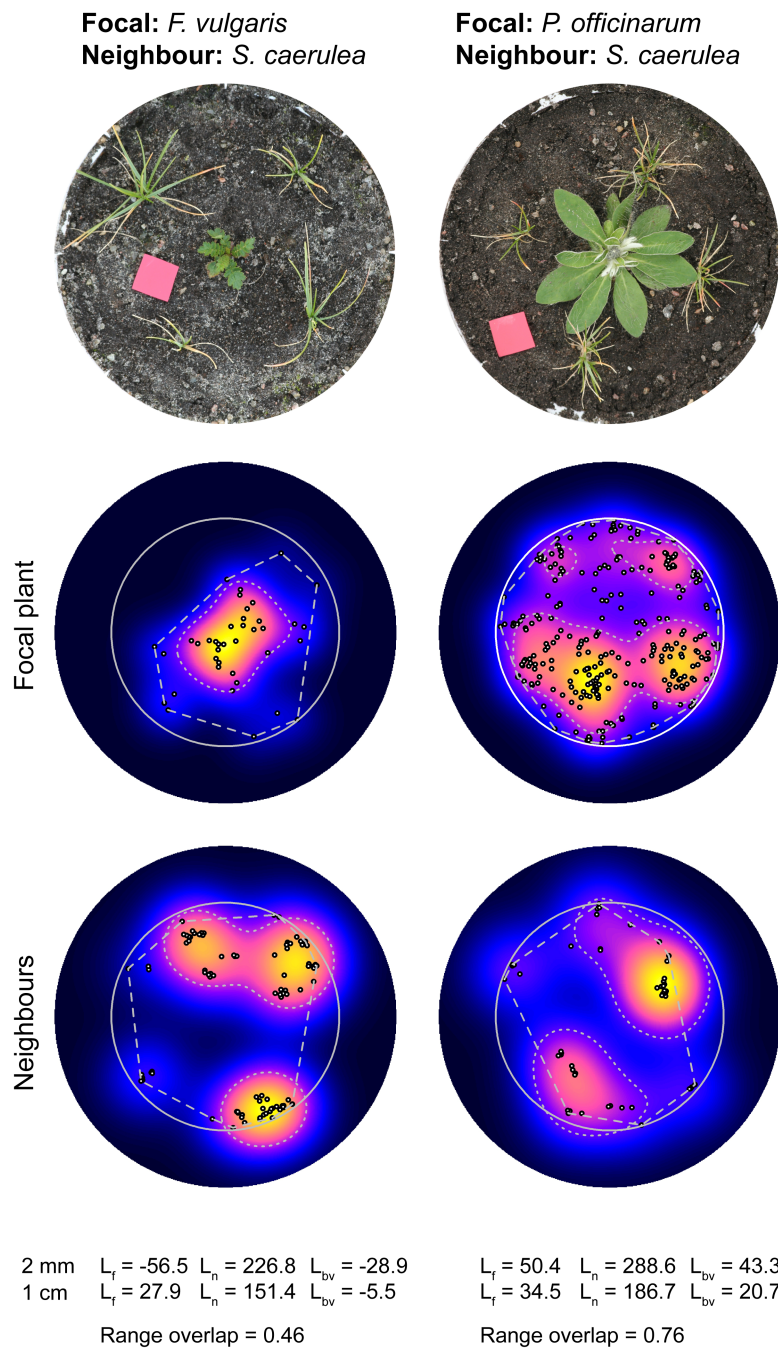
706

707 **Table 2.** Results of linear mixed models examining the effects of species pair identity,
708 neighbour density and soil depth on the size of focal plant foraging ranges in species
709 mixtures (expressed as minimum convex polygon, MCP or 50% kernel core area) and
710 home range overlap between focal and neighbour plants in species mixtures (expressed
711 as the overlap between total kernel volumes). F-values and their significance are shown.
712 \$ $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. df – degrees of freedom. Residual degrees
713 of freedom were 103 for range size models and 73 for range overlap models (based on
714 Kenward-Roger approximations).

	df	Range size		Range volume overlap
		MCP	Core area	
Species (S)	7	40.4 ***	30.6 ***	3.2 **
Neighbour density (N)	1	8.2 **	0.03	27.6 ***
Depth (D)	1	0.07	7.2 **	6.0 *
S:N	7	2.5 *	0.53	3.5 **
S:D	7	1.2	1.8	2.1 \$
N:D	1	4.5 *	1.1	1.2
S:N:D	7	1.2	0.71	1.4

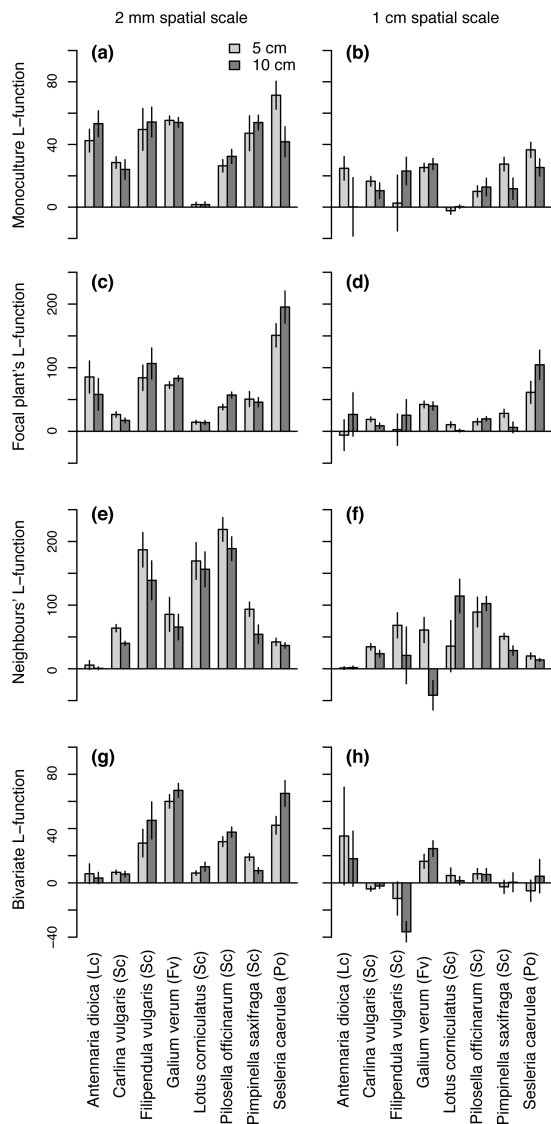
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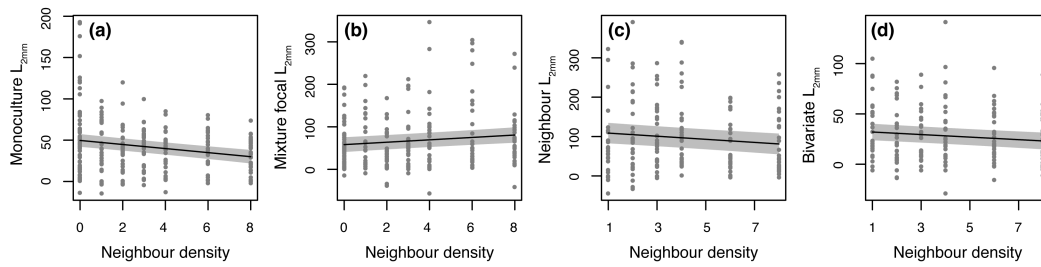
718 **Figure 1.** Examples of root spatial distributions in species mixtures with a focal plant
 719 located in the centre and four neighbours located at 2/3 of the pot radius from the
 720 centre. Yellow colours indicated the highest and dark blue the lowest root densities.
 721 Root locations are shown with grey dots; the solid rings indicate the central area of pots,
 722 where root locations were recorded (2/3 of the pot radius); the dashed lines show
 723 minimum convex polygon (MCP) home ranges; and dotted lines shows the 50% kernel

724 core areas. Roots are less spatially aggregated (as indicated by lower values of the L-
725 functions) and home range overlap is smaller in the mixture of *Filipendula vulgaris* and
726 *Sesleria caerulea* than in the mixture of *Pilosella officinarum* and *Sesleria caerulea*. L_f , L_n
727 and L_{bv} refer to L-functions for intraspecific root distribution of the focal plant and
728 neighbours and bivariate L-function for interspecific distribution, respectively.



729

730 **Figure 2.** Root spatial distribution (L-function) in eight species monocultures (a-b) and
 731 mixtures (c-h) at two soil depths (5 cm and 10cm) and two spatial scales (2 mm and 1
 732 cm). Intraspecific root distributions of the focal plant (c-d), neighbouring plants (e-f)
 733 and interspecific root associations (g-h) calculated as a bivariate L-function are shown.
 734 Predicted values \pm SE at the density of four neighbours are shown. Positive values
 735 indicate root spatial aggregation and negative values indicate spatial segregation. The
 736 names of focal species are shown in full, followed by the abbreviations of neighbouring
 737 species in parentheses (Lc – *Lotus corniculatus*; Sc – *Sesleria caerulea*; Fv – *Filipendula*
 738 *vulgaris*, Po – *Pilosella officinarum*).



739

740 **Figure 3.** Overall effect of neighbour density on root spatial distributions (L-functions)

741 at 2 mm spatial scale. Intraspecific root distributions of all plants in species

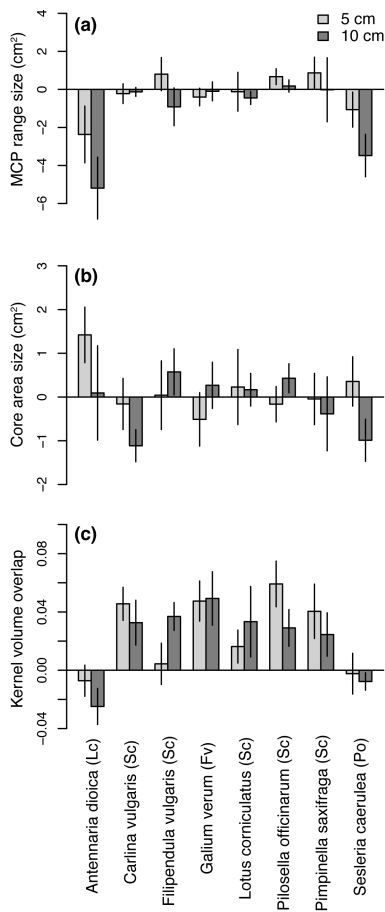
742 monocultures (a), the focal plant (b) and neighbouring plants (c) in species mixtures,

743 and interspecific root associations calculated as a bivariate L-function (d), are shown.

744 Regression lines and 95% confidence intervals obtained from linear mixed models, with

745 species identity and pot included as random factors, are shown.

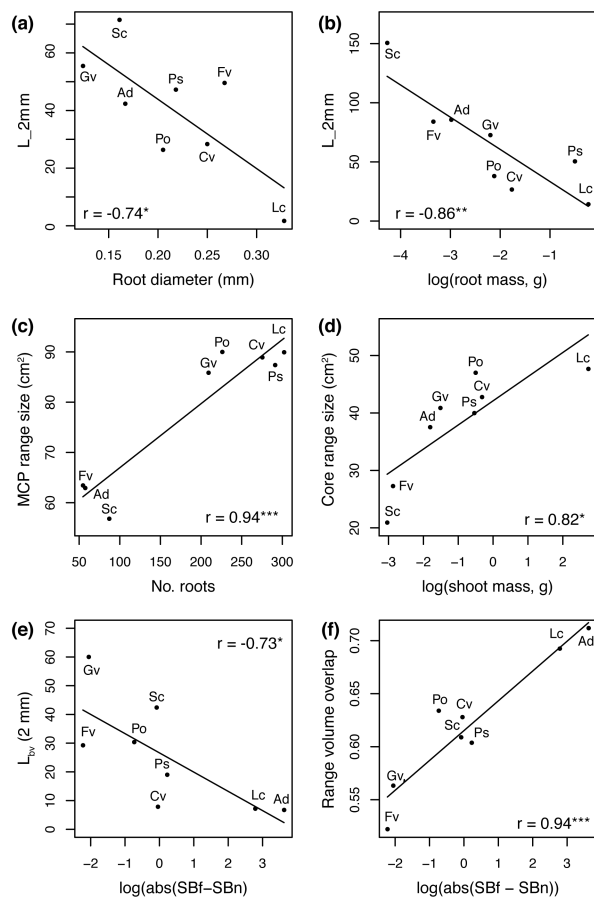
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747

748 **Figure 4.** Changes in focal plant home range size and overlap with increasing neighbour
 749 density in eight species mixtures at two soil depths (5 cm and 10cm). Home ranges were
 750 calculated as an area inside a minimum convex polygon (a) or 50% kernel core area (b).
 751 Home range overlap was calculated based on total kernel volume, with the value of 1
 752 indicating full overlap (c). Slopes \pm SE from a regression of home range against
 753 neighbour density are shown. Positive values indicate an increase and negative value a
 754 decrease in range size or overlap with increasing neighbour density. The names of focal
 755 species are shown in full, followed by the abbreviations of neighbouring species in
 756 parentheses (Lc – *Lotus corniculatus*; Sc – *Sesleria caerulea*; Fv – *Filipendula vulgaris*, Po
 757 – *Pilosella officinarum*).

758



759

760 **Figure 5.** Correlations between root spatial patterns and plant traits. a) Root
 761 aggregation at 2mm spatial scale in species monocultures and root diameter; b) focal
 762 plant's root aggregation at 2mm spatial scale and root mass in species mixtures; c)
 763 minimum convex polygon home range and total number of roots recorded for the focal
 764 plant in species mixtures; d) 50% kernel core area and shoot mass of focal plants in
 765 species mixtures; absolute difference in shoot mass between focal plant and neighbours
 766 in species mixtures and e) interspecific root aggregation at 2 mm spatial scale,
 767 calculated as bivariate L-function, and f) total kernel volume overlap. All values
 768 represent predicted means at mean neighbour density (four neighbours) at soil depth of
 769 5cm. Focal species names are indicated with initial letters for genus and species names.
 770 Pearson correlation coefficients and their significance are shown ($\$ P < 0.1$, $* P < 0.05$,
 771 $*** P < 0.001$).