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

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Author contributions

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

Data accessibility

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

Abstract

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

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

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

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

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

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

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

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

Materials and methods

Experimental design and measurements

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

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

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

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

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

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).

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

Interspecific root spatial distribution

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

Home range size and overlap

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

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

Statistical analysis

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

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

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

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

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

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

Results

Root spatial patterns in species monocultures

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

Root spatial patterns, range size and overlap in species mixtures

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

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

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

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

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

Relationship between root spatial patterns and plant traits

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

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

Discussion

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

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

Root spatial patterns in species monocultures

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

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

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

Root spatial patterns and range overlap in species mixtures

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

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

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

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

Conclusions

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

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TABLES AND FIGURES

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

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

Figure 1. Examples of root spatial distributions in species mixtures with a focal plant located in the centre and four neighbours located at 2/3 of the pot radius from the centre. Yellow colours indicated the highest and dark blue the lowest root densities. Root locations are shown with grey dots; the solid rings indicate the central area of pots, where root locations were recorded (2/3 of the pot radius); the dashed lines show 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.

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

Figure 3. Overall effect of neighbour density on root spatial distributions (L-functions) at 2 mm spatial scale. Intraspecific root distributions of all plants in species monocultures (a), the focal plant (b) and neighbouring plants (c) in species mixtures, and interspecific root associations calculated as a bivariate L-function (d), are shown. Regression lines and 95% confidence intervals obtained from linear mixed models, with species identity and pot included as random factors, are shown.

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

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