Running Title: Facilitation and root positioning

Root positioning and trait shifts in *Hibbertia racemosa* as dependent on its neighbour’s nutrient-acquisition strategy

Patrícia de Britto Costa¹,²*, Christiana Staudinger¹,⁴, Erik J. Veneklaas¹, Rafael S. Oliveira¹,³, Hans Lambers¹

¹ School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, Perth 6009, Australia
² Programa de Pós Graduação em Biologia Vegetal Institute of Biology P.O.Box: 6109, University of Campinas – UNICAMP 13083-970, Campinas, SP, Brazil.
³ Departamento de Biologia Vegetal, Institute of Biology P.O.Box: 6109, University of Campinas – UNICAMP 13083-970, Campinas, SP, Brazil.
⁴ Present address: Rhizosphere Ecology and Biogeochemistry Group, Institute of Soil Sciences, University of Natural Resources and Life Sciences, Vienna, Konrad-Lorenz Strasse 24, 3430 Tulln

ORCID
PdBC: 0000-0002-1445-2900
CS: 0000-0003-0606-9407
EV: 0000-0002-7030-4056
RSO: 0000-0002-6392-2526
HL: 0000-0002-4118-2272

Abstract
Nutrient-poor ecosystems globally exhibit high plant diversity. One mechanism enabling species coexistence in such ecosystems is facilitation among plants with contrasting nutrient-acquisition strategies. The ecophysiological processes underlying these interactions remain poorly understood. We hypothesised that root positioning plays a role between sympatric species in nutrient-poor vegetation. We investigated how growth traits of the focal mycorrhizal
non-cluster rooted *Hibbertia racemosa* change when grown in proximity of non-mycorrhizal *Banksia attenuata*, which produces cluster roots that increase nutrient availability, compared with growth with conspecifics. Focal plants were placed in the centre of rhizoboxes, and we assessed biomass allocation, root system architecture, specific root length and leaf nutrient concentration. When grown with *B. attenuata*, focal plants decreased root investment, increased root growth towards *B. attenuata*, and positioned their roots near *B. attenuata* cluster roots. Specific root length was greater, and the degree of localised root investment correlated positively with *B. attenuata* cluster-root biomass. Total nutrient contents in the focal individuals were greater when grown with *B. attenuata*. Focal plants directed their root growth towards the putatively facilitating neighbour’s cluster roots, modifying root traits and investment. Preferential root positioning and root morphological traits play important roles in positive plant-plant interactions.

Keywords: biotic interactions; cluster roots; carboxylates; facilitation; neighbourhood effects; plant-plant interactions; root aggregation; root spatial patterns; root system architecture; specific root length.

Correspondence
* Corresponding author,
  e-mail patricia.britto.costa@gmail.com
Introduction

The Southwest Australian Floristic region (SWAFR) features among the 36 global hotspots of biodiversity for conservation priorities (Myers, Mittermeier, Mittermeier, da Fonseca & Kent 2000; Williams et al. 2011; Noss et al. 2015). The SWAFR harbours 8379 native vascular plant taxa, of which 47% are endemic to the region (Gioia & Hopper 2017). This represents 2.7% of all biodiversity of vascular plants in the world (Christenhusz & Byng 2016).

This diversity is contained in a nutrient-poor environment, an OCBIL (old, climatically-buffered, infertile landscape), which has extremely nutrient-impoverished soils, especially low in phosphorus (P) (Hopper, Lambers, Silveira & Fieldler 2021). In addition to a high plant species diversity, we find a high diversity of nutrient-acquisition strategies (Zemunik, Turner, Lambers & Laliberté 2015), including not only species that have associations with mycorrhizal fungi but also a range of non-mycorrhizal species that are very efficient in mining nutrients by chemically altering the rhizosphere (Lambers et al. 2014).

In these P-impoverished habitats, we find nutrient-acquisition strategies that differ from those in young, often disturbed fertile landscapes (YODFLs) (Lambers, Brundrett, Raven & Hopper 2010). Species that are highly efficient at mining nutrients sorbed to soil particles, especially P, such as species with cluster roots (Lambers, Raven, Shaver & Smith 2008), exude a large amount of carboxylates that make nutrients sorbed to soil particles available for plant uptake by ligand exchange (Shane & Lambers 2005a). On the other hand, species that have a scavenging strategy, through associations with mycorrhizal fungi, become less competitive in these nutrient-poor habitats, because the P concentration in the soil solution is too low (Parfitt 1979). Thus, in these P-impoverished plant communities, the proportion of species with a nutrient-mining strategy is greater than in YODFLs (Zemunik et al. 2015). But what are the mechanisms that contribute to this high diversity, and how is this maintained?
In the OCBIL theory, Hopper (2009) highlights mechanisms that contributed, over long time scales, to maintaining and generating high diversity in these environments. One of these mechanisms is nutritional specialisation, and the predominant process driving species coexistence is a shift in prevalence from competitive interactions to facilitative interactions (when the presence of one plant enhances a neighbour’s fitness and may occur in concert with negative, positive, or neutral reciprocal responses from neighbours, sensu Callaway 2007), as postulated by the stress gradient hypothesis (Bertness & Callaway 1994). In severely P-impoverished soils, the costs of P acquisition are very high (Raven, Lambers, Smith & Westoby 2018). This might lead to a trade-off in plant investment among different P-acquisition strategies (Ryan et al. 2012). Thus, species that usually rely on associations with mycorrhizal fungi become deprived of nutrients, slowing down their growth rate, thus giving space and opportunities to species with more effective strategies to establish. However, positive interactions with species of contrasting nutrient-acquisition strategies may enable them to persist in these environments. The existence of such positive interactions has been demonstrated in glasshouse studies, involving species with either cluster roots or mycorrhizal associations (Muler, Oliveira, Lambers & Veneklaas 2014; Teste, Veneklaas, Dixon & Lambers 2014). It has also been shown in the field, involving species with either P-mobilising dauciform roots or mycorrhizas (Yu et al. 2020b; Yu, Li, Xiao, Lambers & Li 2020a). Although the outcome of facilitation between species with different nutrient-acquisition strategies has been reported in several studies as the biomass increase in the species relying on facilitation (Cu, Hutson & Schuller 2005; Muler et al. 2014), there are few studies investigating the processes involved in positive species interactions (Yu et al. 2020a). The study of nutrient foraging in plants helps us link the outcome (i.e. biomass increase) with finer-scale mechanisms (i.e. root positioning and root anatomy). Root growth and root positioning are plastic responses to an array
of cues in the soil, including nutrient distribution in the soil, microorganisms and neighbouring species (Cahill & McNickle 2011), resulting in non-uniform root distributions in soil around the root axis (Zhang et al. 2020).

Neighbouring species can affect neighbour root positioning, since they represent non-nutritional cues, affecting overall root spatial allocation in soil (Cahill et al. 2010). A neighbour can affect root placement simply because of spatial constraints, but also chemically, by root-derived compounds (Semchenko, Saar & Lepik 2014; Waters, Soini, Novotny & Watson 2016). In terms of root positioning, a plant can have three basic responses to a neighbour: no response, avoidance and aggregation. The first one occurs when the root placement is independent of presence of neighbour or neighbour identity. This pattern occurs when a species has limited capacity to detect or respond to neighbour presence, or its response does not differentiate self and neighbouring roots (Semchenko, John & Hutchings 2007). The avoidance behaviour is an under-mixing characterised by suppression of root growth in the proximity of neighbouring roots (Schenk, Callaway & Mahall 1999). Finally, aggregation occurs when roots over-mix, and is characterised by an increase in root proliferation (Bartelheimer, Steinlein & Beyschlag 2006). However, these foraging patterns can also lead to changes in root traits such as specific root length (SRL), which may vary to enhance either exploration or absorption of nutrients (Yu et al. 2020b).

The foraging pattern can lead to changes in overall plant investment. For example, in the case of root aggregation, an overproduction of roots is common which may come at a cost such as decreased root growth in unoccupied soil patches, or an increase in root/shoot ratio, thus a reduced fitness (Padilla et al. 2013). However, if we consider a putative facilitative neighbour with a different (complementary) nutrient-acquisition strategy, the root aggregation might result in a greater nutrient acquisition, and likely growth, maintaining root/shoot growth. Also,
to maintain root/shoot investment, plants may alter their specific root length by spending resources, while foraging for nutrients, but saving resources by producing more thin absorptive roots when reaching a nutrient-rich patch. This change in root/shoot investment or specific root length can lead to significant changes in competitive ability, and thus enable species co-existence (Semchenko, Lepik, Abakumova & Zobel 2018).

The aim of this study was to build on previous work, where a clear benefit in terms of growth of the facilitated species was demonstrated in a glasshouse study (Muler et al. 2014) and field observations showing neighbour roots intermingling with cluster roots (Teste, Dixon, Lambers, Zhou & Veneklaas 2020). Thus, we investigated whether the presence of a putative facilitating non-mycorrhizal cluster-root-bearing neighbouring species, *Banksia attenuata*, increases growth and leaf nutrient content of *Hibbertia racemosa*, a mycorrhizal species without cluster roots. If so, does *H. racemosa* change its root architecture in response to this positive interaction? Additionally, do these changes in root architecture cause increased aggregation with the cluster roots of *B. attenuata*? We hypothesised that *H. racemosa* would display increased shoot growth when co-occurring with *B. attenuata*, as nutrient availability would be greater with cluster roots being efficient at solubilising soil nutrients, especially P. This greater nutrient availability around cluster roots would influence *H. racemosa’s* root positioning, making it grow closer to the roots of *B. attenuata*.
Materials and Methods

Experimental species

_Hibbertia racemosa_ (Endl.) Gilg seedlings were acquired from Australian Native Nursery in Perth, Australia. _Banksia attenuata_ R.Br. seeds were collected in the field, in Jurien Bay, Western Australia, and germinated in a glasshouse, UWA, Perth, in February. The species choice was based on their co-occurrence in their natural habitat, with one of them being non-mycorrhizal, producing cluster roots (_B. attenuata_), and the other being mycorrhizal, but without cluster roots (_H. racemosa_). Both are common in the Southwest Botanical Province of Western Australia.

_Banksia attenuata_ and _H. racemosa_ plants differed in size, with shoot biomass of _H. racemosa_ being three times that of _B. attenuata_ (Supplementary Fig. 1). This was because we bought _H. racemosa_ from a nursery and _B. attenuata_ was grown from seeds to ensure a rapid production of cluster roots due to a low-P regime. However, to minimise the effect of initial plant size in the rhizobox, we trimmed the roots to the same size to ensure all individuals had a similar timespan to occupy the rhizobox volume.

Experimental design

We used rhizoboxes with inner dimensions of 60 x 40 x 3 cm, where 60 cm is the vertical dimension. During the experiment, rhizoboxes were placed at a 60° angle relative to the horizontal, with the transparent 60 x 40 cm acrylic sides facing downward and darkened with black covers, so the roots grew towards and along the transparent side and light did not interfere with root development. We filled all rhizoboxes (5.0 L) with dried soil (top 80 mm) collected from a _Banksia_ woodland on a Bassendean dune along the Jurien Bay chronosequence in Western Australia, which consists of oligotrophic sand; it is severely nutrient impoverished and strongly leached (Laliberté _et al._ 2012).
We planted the individuals of *B. attenuata* and *H. racemosa* in a replacement series design with proportions of 2 *B. attenuata* and 1 *H. racemosa* in the BHB treatment (n = 7), 1 *B. attenuata* and 2 *H. racemosa* in the BHH treatment (n = 7) and 0 *B. attenuata* and 3 *H. racemosa* in the HHH treatment (n = 7). To transplant *H. racemosa* and *B. attenuata* seedlings into the rhizoboxes, we made holes of the same size in the soil to make sure they started with the same root volume; for this, we had to trim *H. racemosa* roots. In the BHH treatment we planted the *B. attenuata* individuals at the left side of the rhizobox in five boxes and at the right side in another five boxes (3 boxes were excluded because one or more individuals died), to avoid any effects of planting side. However, for the sake of data analysis, we considered all *B. attenuata* individuals in the BHH treatment were on the left side. The plants were grown in a naturally-lit greenhouse from June to October 2017 at the University of Western Australia, Perth (31°59'03.4"S 115°49'10.6"E), with mean maximum and minimum temperatures of 24.6 and 12.7°C.

**Root growth measurements and analysis**

At harvest, we divided the rhizoboxes into six columns and three rows, comprising 18 sections with the same soil volume. In each section, the roots were washed and split according to species and individuals. Roots of *B. attenuata* and *H. racemosa* differ in colour, and they could therefore be separated, even though they intermingled. *Hibbertia racemosa* roots could be traced from their tips to their shoot base; this allowed the separation of roots of conspecific individuals.

All separated roots were kept in sealed plastic bags with a humid paper towel inside a cool room, so root integrity was maintained until subsequent scanning. For each *B. attenuata* plant, we also counted the number of cluster roots and determined their dry weight. Our approach made it possible to assess spatial root allocation in a directional and precise manner.
We scanned all roots using a V800 Epson professional scanner (Long Beach, CA, USA) at 400 dpi per mm in professional mode, greyscale. The images were analysed in WinRHIZO software (v2009, Regent Instrument, Quebec, QC, Canada) to assess root length and diameter. We dried the samples and weighed them on an analytical balance, to calculate specific root length and root investment. The calculated horizontal root asymmetry index (Hra) was

$$Hra = \frac{Lr - Rr}{Tr}$$

where $Lr$ is the focal species root dry weight in the left half of the rhizobox ($B.\ attenuata$ side in the BHH treatment), $Rr$ is the focal species root dry weight in the right half of the rhizobox, and $Tr$ is the focal species total root dry weight. Thus, the Hra index indicates root weight allocation asymmetry between the left and right side, positive values indicating greater root allocation to the left side and negative values indicating a greater allocation to the right side. Additionally, we calculated the vertical root allocation index (Vra) as

$$Vra = \frac{\text{top}20 - \text{bottom}60}{Tr}$$

where $\text{top}20$ is the focal species root dry weight in the rhizobox top 20 cm, $\text{bottom}60$ is the focal species root dry weight from 20 to 60 cm depth, and again $Tr$ is the focal species’ total root dry weight. Higher values in Vra indicate a greater proportion of roots allocated to the top 20 cm of the rhizobox, with negative values indicating less than 50% of root biomass was allocated to the top 20 cm.

We assessed rooting width (Rw) among treatments by comparing the ratio of root biomass in the outer two left columns (Lrw) and the two central columns (Crw), with the equation $Rw = \frac{Lrw}{Crw}$. To further illustrate changes in root allocation among treatments, we represented the average root dry weight percentage in each section in a graph, indicating whether the growth was towards heterospecific ($B.\ attenuata$) or conspecific ($H.\ racemosa$) individuals. Finally, to test if root allocation of $H.\ racemosa$ was correlated with cluster-root biomass, we selected only the outer two sections of the top and middle rows, that had at least one cluster root and assessed a possible correlation between focal plant root dry weight and cluster-root dry weight in these sections.
We harvested shoots, dried these for 48 h at 60°C, separated stems from leaves, and measured their dry weight. The dry leaf material was then ground, and subsamples digested in hot concentrated nitric acid: perchloric acid (HNO₃: HClO₄; 3:1) (Malavolta, Vitti & De Oliveira 1997), to determine the concentrations of P, manganese (Mn), copper (Cu), aluminium (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), molybdenum (Mo), sodium (Na), sulfur (S), and zinc (Zn) for both species by atomic absorption spectrometry (Perkin Elmer 5300 DV ICP-OES, Waltham, Massachusetts, USA).

**Data analysis**

All statistical analyses were performed in R (version 3.5.1 (R Core Team, 2018)). Before each analysis of variance, data and residuals were tested for normality using the Shapiro-Wilk test and Bartlett’s test for homogeneity of variances. As shoot and root dry weight and root shoot ratio of *H. racemosa* met ANOVA assumptions, we analysed these as response variables in distinct one-way ANOVA tests, followed by Tukey HSD post-hoc test. For nutrient analyses, we performed the same analyses. Specific root length did not meet ANOVA assumptions; thus, we analysed the variance between groups, using the non-parametric Kruskal-Wallis test, followed by Wilcoxon rank-sum test. To analyse the relation of *H. racemosa* root dry weight and *B. attenuata* cluster-root dry weight, we used a linear regression model (Lm), after Log transformation to normalise the data.

Hra, Vra and Rb did not meet ANOVA assumptions; thus, we analysed the variance between groups using the non-parametric Kruskal-Wallis test, followed by Wilcoxon rank-sum test. Root biomass for each rhizobox section was also standardised by converting it to a percentage of the total root biomass of that replicate, in order to assess root distribution independent of total biomass.
Results

Growth of *H. racemosa* and *B. attenuata*

In order to understand the effect of neighbour identity on *H. racemosa* biomass allocation patterns, we evaluated focal *H. racemosa* shoot and root biomass after five months of growth with only conspecific pairs, or with one or two *B. attenuata* individuals on the side. Although focal species did not differ in total biomass production when growing with *B. attenuata* individuals \((P = 0.97)\), we observed changes in the allocation pattern between roots and shoots. Focal species invested slightly less (9.7%) in roots when planted with one *B. attenuata* plant, and significantly less (69%) when planted with two *B. attenuata* plants \((P = 0.016)\). In contrast, focal plants showed greater shoot biomass when planted together with one (13%) or two *B. attenuata* plants (22%) than when grown only with conspecific pairs, but this was not significant \((P = 0.574)\). Taken together, this resulted in a significantly lower root/shoot ratio in *H. racemosa* when planted with two *B. attenuata* plants (Figure 1).

*Hibbertia racemosa* root system architecture and other traits

Root positioning of the focal *H. racemosa* changed according to neighbour identity, from low root overlapping, when planted only with conspecifics, to root aggregation, with intensive overlapping, when planted with *B. attenuata*. The root asymmetry index, defined as inequality of root distribution between both sides of the focal plant in the rhizobox, was greater when the focal plant was grown with one *B. attenuata* individual \((P = 0.001)\). Root asymmetry ranged from -0.12 to +0.17 in the treatment with only conspecifics, from 0.12 to 0.52 in the treatment with one *Banksia* on one side, and from -0.56 to +0.25 in the treatment with one *Banksia* on each side, indicating greater root allocation towards the *B. attenuata* side when planted with one *B. attenuata* and a greater variation in root asymmetry when grown with a *B. attenuata* plant at both sides (Figure 2a).
The rooting width, i.e. the proportion of focal *H. racemosa* root mass allocated to the rhizobox outer two lateral sections, increased when the neighbour plant was *B. attenuata*, compared with when *H. racemosa* was the neighbour (Figure 2c) (Kruskal-Wallis chi-squared = 12.576, P = 0.001, df = 2) (n=7). Moreover, the outermost lateral sections had no focal *H. racemosa* roots at all when the neighbour was a conspecific. This pattern of greater lateral root growth of the focal plant was also accompanied by a tendency of a greater percentage of roots in the top 20 cm of the soil compared with that in the deeper layers of the rhizobox, in both treatments with *B. attenuata*, although Vra was not significantly different (Figure 2b).

Beyond root asymmetry, we observed that focal *H. racemosa* plants aggregated their roots with *B. attenuata* roots, sometimes intermingling (Figure 3a-b), and in some cases growing into a *B. attenuata* cluster rhizosphere (Figure 3c-d). In order to evaluate the effect size of these interactions, we correlated the cluster-root dry weight with focal *H. racemosa* root dry weight in each section, and found that the degree of localised root investment correlated positively with *B. attenuata* cluster-root biomass (Figure 4); thus the greater the cluster-root dry weight, the greater the *H. racemosa* root positioning in that same rhizobox section.

Finally, we aimed to understand if the changes in root investment and architecture were accompanied by changes in root traits, in particular SRL and root diameter. The SRL of focal *H. racemosa* was greater when grown with one or two *B. attenuata* plants, but this was only statistically significant for the BHB treatment (P=0.045) (Figure 5a). This was accompanied by a smaller root diameter in the focal plant as well, being significantly lower in the treatment BHH and BHB (P=0.045) (Figure 5b).

**Leaf nutrient concentration and content in *H. racemosa***

The presence of a putative facilitator has previously been shown to increase nutrient concentrations in the leaves of co-occurring species; therefore, we measured nutrient concentrations and calculated nutrient contents in the leaves to track this possible outcome. Leaf P and Cu
content was significantly greater in the focal plants grown with two *B. attenuata* plants (Figure 6). In contrast, contents of all other elements in the focal plants were similar in all treatments (one-way ANOVAs: for Mn, Al; Ca; Fe; K; Mg; Mo; S and Zn, *P* >0.05). Copper was the only leaf nutrient, whose concentration differed in focal *H. racemosa* plants when grown with one or two *B. attenuata* (Supplementary Fig. 2). The presence of *B. attenuata* did not affect the concentration of any other elements in the leaves (one-way ANOVAs: for P; Mn; Al; Ca; Fe; K; Mg; Mo; Na; S and Zn, *P* >0.05).

**Discussion**

In the present study, we investigated root responses in a positive interspecific interaction and showed that the presence of a cluster-root bearing neighbour (*B. attenuata*), as a putative facilitating species, led to differences in root investment and architecture in the focal *H. racemosa*. When grown with *B. attenuata*, focal plants extended their roots towards *B. attenuata* roots, with a tendency to allocate a greater proportion of their roots in the top 20 cm of the soil. Additionally, the specific root length of the focal *H. racemosa* plants was greater when grown with *B. attenuata*, and focal plant root positioning correlated with the presence of cluster roots of *B. attenuata*. Finally, leaf P and Cu content were greater in focal species when grown with *B. attenuata*, and only leaf Cu concentration was greater.

**Growth and biomass allocation patterns of *H. racemosa***

Clear benefits in terms of shoot growth of the facilitated species have been shown before in experiments with complementary nutrient-acquisition strategies (Cu *et al.* 2005; Muler *et al.* 2014). We went beyond that, and specifically assessed the effect of conspecific and heterospecific interactions on the focal species root growth. Focal species, *H. racemosa*, when grown with *B. attenuata*, sense neighbour’s presence, responding with root growth towards *B. attenuata’s* roots, or, alternatively, could invest in root growth in all directions, until reaching
patches of increased nutrient availability in the soil due to cluster-root activity. Once reaching the cluster rhizosphere, *H. racemosa* allocated more root biomass in this soil patch to exploit nutrients available, increasing root mixing. Further studies are necessary to understand if there is any sensing involved in root growth direction in these positive root-root interactions, as shown for other studies in clonal plants (Waters & Watson 2015), and if so, identify its origin in these interspecific belowground interactions (van Dam & Bouwmeester 2016).

The focal plants significantly differed in root/shoot ratio, when growing with two *B. attenuata* plants, indicating that biomass allocation changed, depending on neighbour identity. Optimal resource allocation to shoot/root investment can enhance plant success in a community (Bloom, Chapin III & Mooney 1985). In the nutrient-poor Bassendean dune sand, in which *H. racemosa* grows as a non-dominant species (Zemunik, Turner, Lambers & Laliberté 2016), the presence of the putative facilitative species changed its root-shoot investment. This contributed to decrease overall root-shoot asymmetry in biomass allocation to compensate nutrient limitation (Kembel, De Kroon, Cahill & Mommer 2008) (Figure 1). Thus, the performance of individuals to persist in a community would depend on multiple community assembly factors, including neighbour abundance and frequency, increasing the probability of facilitation interactions when *H. racemosa* is moderately abundant where Proteaceae are dominant (Freestone 2006).

**Root system architecture and other traits of *H. racemosa***

Root symmetry

Neighbour identity was an important factor determining focal plant root placement in the rhizoboxes (Figure 2a-b). We interpret this as preferential positioning of roots where nutrient availability increased, potentially involving signals from the rhizosphere (Waters & Watson 2015), caused by the exudates released in the cluster rhizosphere of *B. attenuata* (Shane & Lambers 2005a). These patches of high nutrient availability presumably are a preferred spot
for nutrient acquisition, particularly for nutrient-limited neighbours such as *H. racemosa* that do not share the same nutrient-acquisition strategy. The signalling for guiding root placement can come from the neighbour root exudates (Bais, Weir, Perry, Gilroy & Vivanco 2006; Semchenko *et al.* 2014), volatile organic compounds (Waters *et al.* 2016), or even the rhizosphere microbiome (Mommer, Kirkegaard & van Ruijven 2016). Thus, when co-occurring with a cluster-root bearing species, *H. racemosa* could allocate its root growth to preferred spots, preferentially positioning roots, instead of random root growth.

When planted with two *B. attenuata* plants, the root asymmetry presented greater variation (Figure 2a), indicating that in some cases there was a preference for one side. Since *H. racemosa*’s root weight correlated positively with cluster-root presence (Figure 4), the asymmetry is likely due to differences in cluster root numbers that each *B. attenuata* individual produced, their exudation rate and when they were formed, influencing *H. racemosa* root growth differently in each rhizobox side, contributing to greater variation.

Root traits

Beyond root asymmetry, we observed that focal *H. racemosa* root presence was correlated with the presence of *B. attenuata* cluster roots in the same section (Figure 4). However, the presence of cluster roots only explained 40% of root positioning. This is likely because the non-cluster roots of *B. attenuata* roots also release carboxylates (Roelofs, Rengel, Cawthray, Dixon & Lambers 2001; Shi, Strack, Albornoz, Han & Lambers 2020), solubilising soil nutrients. We also highlight *H. racemosa* root positioning in specific sections in the rhizobox, with a tendency of investing a greater root biomass proportion in the top 20 cm of the soil (Figure 2b). In natural conditions, in nutrient-poor soils such as the Bassendean dunes, *B. attenuata* produces cluster roots in the topsoil, where nutrient concentrations are higher (Turner & Laliberte 2015). In the rhizobox, *B. attenuata* also produced the cluster roots in the topsoil, triggering the focal plants to invest proportionally more root biomass in the topsoil as well.
This differential root investment when *H. racemosa* grew with *B. attenuata* individuals shows that interspecific interactions between species with contrasting nutrient-acquisition strategies affect root partitioning in the soil, which in turn affects species coexistence. Neighbour identity had an effect on the specific root length (Figure 5a). Root proliferation in nutrient-rich patches is usually accompanied by an increase in SRL (Robinson 1994). The increase in SRL is negatively correlated with root diameter, indicating thinner roots that have a higher surface area/volume ratio, and thus faster absorption rate per unit root weight (Kong, Wang, Kardol & Zeng 2015). *Hibbertia racemosa* roots were thicker when growing with only conspecifics; thus, we infer that when *H. racemosa* individuals were in the explorative mode foraging for nutrients in all directions, without signals from nutrient-rich patches, they maintained thick foraging roots. In contrast, when grown with *B. attenuata* on one or two sides, focal plants, after growing towards nutrient-rich patches, started to produce thinner absorptive roots and exploited a nutrient patch (Hodge 2009). Additionally, thinner roots have shorter life spans (McCormack, Adams, Smithwick & Eissenstat 2012) including cluster roots that live only for about three weeks and have a carboxylate exudative burst during only 2-3 days (Shane & Lambers 2005b). Thus, producing thinner absorptive roots with short life spans can be adaptative in *H. racemosa*, since this increases nutrient acquisition with lower root biomass investment in Banksia's rhizosphere.

**Nutrient content and concentration patterns in *H. racemosa***

Although shoot growth was not significantly different, the greater shoot biomass contributed to a higher P and Cu content of focal *H. racemosa* in the presence of Banksia neighbours (Figure 6). In addition, the total root biomass was smaller, especially in the BHB treatment, indicating that P and Cu uptake was more efficient in the presence of Banksia neighbours. Many plant species native exclusively to nutrient-impoverished soils have limited control over P uptake when exposed to high P availability (Lambers *et al.* 2013). However, the distribution of
H. racemosa is not limited to the most nutrient-impoverished habitats. Along the Jurien Bay chronosequence, it also occurs on younger nutrient-rich soils, and we infer that the constant leaf nutrient concentrations of this species in our experiment indicate that H. racemosa plants control their nutrient uptake, maintaining constant leaf nutrient concentration. Leaf P and Mn concentrations were the same in all focal individuals, whilst copper concentration increased when occurring with B. attenuata (Supplementary Fig. 2). This suggests that Mn and P were limiting for H. racemosa growth in the HHH treatment, whereas Cu was available in sufficient amounts, which is similar to findings reported by Muler et al. (2014). Alternatively, the higher P availability, due to cluster-root activity, might lead to more P being invested in shoot growth, in the BHH and BHB treatments. Thus, both Cu and P were mobilised by cluster roots, and the enhanced P availability allowed an increased growth rate, explaining where the greater P uptake was invested. Conversely, the availability of Cu was enhanced, but since it was not limiting for growth, unlike P and Mn, the Cu concentration and content increased with increased growth.

Conclusions
We showed that the presence of a neighbour with a carboxylate-releasing P-acquisition strategy, specifically a cluster-root-bearing species, affected root position of a focal species in nutrient-poor soils. We provide evidence that preferential root positioning close to cluster roots and greater investment in thin roots are key traits for facilitation that can be induced by neighbours. Our results highlight that root interactions in nutrient-poor environments are key components that bear the potential to affect local species diversity through facilitation (Lambers et al. 2018). The present results contribute to our understanding of plant-plant interactions in nutrient-poor habitats, shaping diverse communities, where many species interact at the same time. Future research could focus on characterising the signals that trigger the observed root interactions.
Acknowledgements
This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Brasil – Finance Code 001 (Capes, PhD scholarship to PdBC and Project Grant PVE 88881.068071/2014-01 fellowship to HL, RSO and PdBC), by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), by the Biologia Vegetal post-graduation program at Unicamp and the School of Biological Sciences of the University of Western Australia. R.S.O. was supported by a CNPq–Productivity Fellowship. HL was supported by grants from the Australian Research Council (DP140100148 and DP130100005). CS was supported by an Erwin Schrödinger Postdoctoral Fellowship of the Austrian Science Fund (FWF), grant number J4127.

Conflict of interest
The authors declare no conflicts of interest

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

References


Figures

Figure 1 | (a) Shoot dry weight, (one-way ANOVA; $P = 0.574$, $F = 0.57$, df = 2) (b) total root dry weight (one-way ANOVA; $P = 0.01$, $F = 5.18$, df = 2) and (c) root/shoot ratio (one-way ANOVA; $P = 0.047$, $F = 3.71$, df = 2), (n=7) of focal individuals of *Hibbertia racemosa* in experimental groups of three *H. racemosa* individuals (HHH), two *H. racemosa* individuals with one *Banksia attenuata* plant (BHH), and one *H. racemosa* individual with two *B. attenuata* plants (BHB). Boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, the whiskers represent 1.5*interquartile range and dots represent points outside the extent of 1.5*interquartile range. Symbols are individual samples, and different lowercase letters represent significant differences between soil types following Tukey HSD post-hoc test.
Figure 2 | (a) Horizontal root asymmetry index (Hra) in each treatment, (Kruskal-Wallis chi-squared = 7.061, $P = 0.029$, df = 2) (n=7). (b) Vertical root asymmetry index (Vra) in each treatment, (Kruskal-Wallis chi-squared = 2.9948, $P = 0.223$, df = 2) (n=7). (c) Schematic drawing of rhizoboxes and the focal *Hibbertia racemosa* mean root biomass percentage in each of the 18 rhizobox sections in each treatment (n=7). Each rectangle represents a section in the rhizobox, and the blue colour code represents the mean root mass percentage of focal *H. racemosa* in each section.
Figure 3 | Photographs taken during sampling. (a) two *Hibbertia racemosa* plants next to each other; (b) *H. racemosa* (left) next to *Banksia attenuata* (right). Both images are from the top 20 cm of the root system. (c – d) Detail of *H. racemosa* roots (white, indicated by the black arrows) grown within the rhizosphere of compound cluster roots formed by *B. attenuata*. 
**Figure 4** | Positive relationship between focal *Hibbertia racemosa* root dry weight and the cluster-root dry weight. We included only the outer two sections of the first and middle row that contained cluster roots to minimise the effect of main root positioning in the centre of the rhizobox. Each point represents one section in one rhizobox of treatments BHH or BHB ($F = 10.96$, $P < 0.05$, $R^2 = 0.40$, df = 30).
Figure 5 | Focal plant root traits. (a) Specific root length (Kruskal-Wallis chi-squared = 6.1913, \( P = 0.045 \), df = 2) and (b) root diameter (one-way ANOVA; \( P = 0.45 \), \( F = 17.17 \), df = 2) of focal *Hibbertia racemosa* plants in each treatment, three *H. racemosa* individuals (HHH), two *H. racemosa* individuals with one *Banksia attenuata* plant (BHH), and one *H. racemosa* individual with two *B. attenuata* plants (BHB), (n=7). Superscript letters above each boxplot represent group differences.
Figure 6 | (a) Phosphorus (P) content (one-way ANOVA; $P = 0.012$, $F = 6.76$, df = 2), (b) manganese content (Mn) (one-way ANOVA; $P = 0.463$, $F = 0.83$, df = 2) and (c) copper (Cu) content (one-way ANOVA; $P = 0.031$, $F = 4.69$, df = 2), of the focal Hibbertia racemosa plant in experimental groups of three H. racemosa individuals (HHH), two H. racemosa individuals with one Banksia attenuata plant (BHH), and one H. racemosa individual with two B. attenuata plants (BHB), (n=7).
**Supplementary Figure 1** | Biomass dry weight of both species at the end of the experiment. Boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, the whiskers represent 1.5*interquartile range.
Supplementary Figure 2 | (a) Leaf phosphorus (P) (one-way ANOVA; $P = 0.622$, $F = 0.488$, $df = 2$), (b) manganese (Mn) (one-way ANOVA; $P = 0.744$, $F = 0.301$) and (c) copper (Cu) (one-way ANOVA; $P = 0.004$, $F = 7.361$) concentrations of the focal *Hibbertia racemosa* plant in the experimental groups of three *H. racemosa* individuals (HHH), two *H. racemosa* individuals with one *Banksia attenuata* plant (BHH), and one *H. racemosa* individual with two *B. attenuata* plants (BHB). Boxes represent the interquartile range of the distributions, horizontal dark lines inside them represent the medians, the whiskers represent 1.5*interquartile range and dots represent points outside the extent of 1.5*interquartile range.