REGULAR ARTICLE

Effects of corn (*Zea mays* L.) on the local and overall root development of young rubber tree (*Hevea brasiliensis* Muel. Arg)

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Abstract Understanding better the interactions between root systems in associated crops is significant for basic knowledge in plant science and to help designing cropping systems. Current research on inter-specific root interactions concentrates on static descriptions of the horizontal extension of root systems or on the dynamics of provoked root encounters. This study considers detailed observations of the dynamics of inter-specific root interactions, in the vertical plane, at both the whole root system and the individual root levels. Corn and young rubber trees were grown in association in artificial conditions that excluded the possibility of competition for resources, using rhizoboxes, i.e. thin containers with a transparent wall. The paper presents novel approaches, such as the study of root system growth trajectories, to document root system development in terms of overall growth rate, colonization of soil space and individual root growth patterns. It was found that (i) corn roots developed towards rubber roots until a contact was established, (ii) rubber roots expanded faster and more vertically in association with corn, (iii) the expansion rates of both root systems varied concomitantly and (iv) inter-specific root encounters resulted in reduced elongation rates in both species. Implications of these results for corn/rubber inter-cropping are discussed. This work advocates in favour of a better understanding of under-ground facilitative effects between species. If understood enough to be manipulated, such knowledge might become a powerful tool for the design of more sustainable and efficient cropping systems.

Keywords Below-ground interactions · Root architecture · Trajectories · Inter-cropping · Root development

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Abbreviations
DAS Days after sowing
RLD Root length density

Introduction

A central objective of multi-specific agrosystems, such as inter-cropping and agroforestry, is to sustainably maintain or increase land productivity by optimizing the use of environmental resources (light, water and nutrients) and services (e.g. interactions with soil micro- and macro-fauna) needed for plant growth (Gregory 2006). The diversity of root systems corresponding to a multi-specific vegetation cover offers options to utilize soil and even bedrock resources over an extensive range of lateral and vertical distances (Stone and Kalisz 1991; Calder et al. 1997; Jackson et al. 2000).

It has long been recognized that both intra- and inter-specific interactions between plants trigger plastic behaviours through which their growth patterns, including root development, are substantially modified (Weaver and Clements 1938; Schenk et al. 1999). Recent studies showed that both annual/ annual and perennial/annual intercropping systems, induced alterations of the rooting profiles of both the main and the inter- crop (Li et al. 2006; Mulia and Dupraz 2006).

Within plant communities, the development of adjacent root systems relative to one another appears to result from a concomitance of aggregation, segregation and proliferative behaviours. It has long been recognized that in many species, root systems respond to local nutrient availability by proliferating branches into nutrient-rich patches (Passioura and Wetselaar 1972; Drew; 1975). In the case of nitrogen availability, local root proliferation results from a genetically controlled sensing and signaling of locally high nitrate concentrations, which triggers lateral root initiation and elongation (Zhang and Forde 1998).

Aggregative root placement appears to result from more complex environmental interactions. Although it has remained a predominantly unrecognized phenomenon, many plant species (Bartelheimer et al. 2006) tend to aggregate roots towards their neighbours, even though this is detrimental to root development in soil unoccupied by other plants, indicating the importance of contesting between individuals in relation to resource acquisition (Gersani et al. 2001). Some authors have suggested that in the presence of a competitor, plants might use root aggregation as a defensive reaction to gain or maintain exclusive access to some soil volumes, with direct analogies

with territoriality amongst animal taxa (Schenk et al. 1999; Gersani et al. 2001).

Somewhat contradictorily with what precedes, the literature record also shows that, under limiting environmental conditions, plant root systems preferentially occupy soil volumes that are relatively free of other roots, a process referred to as spatial segregation, which results in limited or the absence of overlap between root systems (Schenk et al. 1999). It has even been argued that root segregation could be further reinforced through allelopathy: plants growing in resource-limited environments or under stress often exhibit higher tissue concentrations of secondary compounds, amongst which allelochemicals, than when growing under more favourable conditions (Grime 1977; Gershenzon 1984; Tang et al. 1995; Aerts and Chapin 2000).

While its precise mechanisms are to be elucidated, evidence of intra- and inter-specific root communication has been reported by several authors. For example, detailed observations of root encounters, showed that a desert shrub species (*Ambrosia dumosa*) can detect and avoid the root systems of con-specific individuals, while another desert shrub species (*Larrea tridentata*) inhibited both con- and allo-specific roots in their vicinity (Mahall and Callaway 1991).

Current knowledge, although still fragmentary, clearly indicates that below-ground interactions between plants involve mechanisms that are particularly difficult and/or tedious to characterize, so that progress with the design of improved agro-systems has been slow, although some general principles are beginning to emerge (Ozier-Lafontaine et al. 1998; Gregory 2006). To design an effective multi-specific agro-system, it is essential to determine the degree of coordination, and the competition or facilitation that ensues, between the rooting habits of the plant species that make up the system's planned biodiversity.

In this respect, this study reports the results of rhizobox experiments carried out to investigate the effects of a candidate annual inter-crop (corn) on rubber root growth. Experiments conducted since the mid-1970s have consistently indicated that the inter-cropping of immature rubber can be both economically and agronomically advantageous (Laosuwan 1996). While it represents a source of income to small farmers during the immature stage of a plantation, it was found to have no detrimental, and under some circumstances, beneficial impacts on the

development of rubber trees (Laosuwan et al. 1988). In this work, young rubber tree seedlings of a clonal variety widespread throughout Southeast Asia and a commercial cultivar of glutinous corn, were grown in combination, under semi-controlled, simplified conditions. The chosen experimental design virtually excluded competition for resources in order to test whether one or both of the associated plants could develop a 'territorial' behaviour (Schenk et al. 1999), independent from foraging for limited resources.

## Materials and methods

Experimental layout

The experiment was conducted at the Faculty of Agronomy field site at Khon Kaen University, Khon Kaen, Thailand (N16° 28′ 15.3″ E102° 48′ 38.6″) from the 5th of March to the 20th of May 2008, until corn ears were ready to be harvested. Root growth of rubber trees and corn were monitored using rhizoboxes, i.e. thin containers with a transparent wall through which roots can be observed at regular intervals (see detailed description below), a simple concept that dates back to at least the early 1900s (e.g. McDougall

1916). Two treatments were compared: rubber tree and corn growing together (Fig. 1) and rubber tree alone. Each treatment was repeated 3 times. A corn alone treatment was not included because this work focused on assessing the effect of annual inter-crops (amongst which corn) on rubber trees and not the reciprocal effects of corn and rubber on one another.

Plant material

Seeds of the glutinous corn (Big White™ 852) were used in the study. Corn seeds were pre-germinated for 3–4 days and subsequently transplanted in the rhizoboxes, 2 cm below the growth medium surface. Care was taken to place the radicule of pre-germinated corn seeds in a position as close to vertical as practically achievable. Buds of *Hevea brasiliensis*, clone RRIM 600, were grafted on illegitimate RRIM 600 rootstock. Bud-grafted plants were grown for 10 months in plastic bags 30 cm high, 10 cm in diameter. During this first nursing period, the taproot was pruned. These young rubber trees were then washed free of soil and nursed in moist vermiculite (Agra-vermiculite, Dutch Greenery Co., Ltd.) for approximately 1 month, in order to encourage root system development prior to transplantation in rhizoboxes.

Fig. 1 Diagrammatic representation of the rhizoboxes. Left: general view with front shutters open. Right: cross section showing the different elements of the system (the vertical and horizontal dimensions are not to scale)
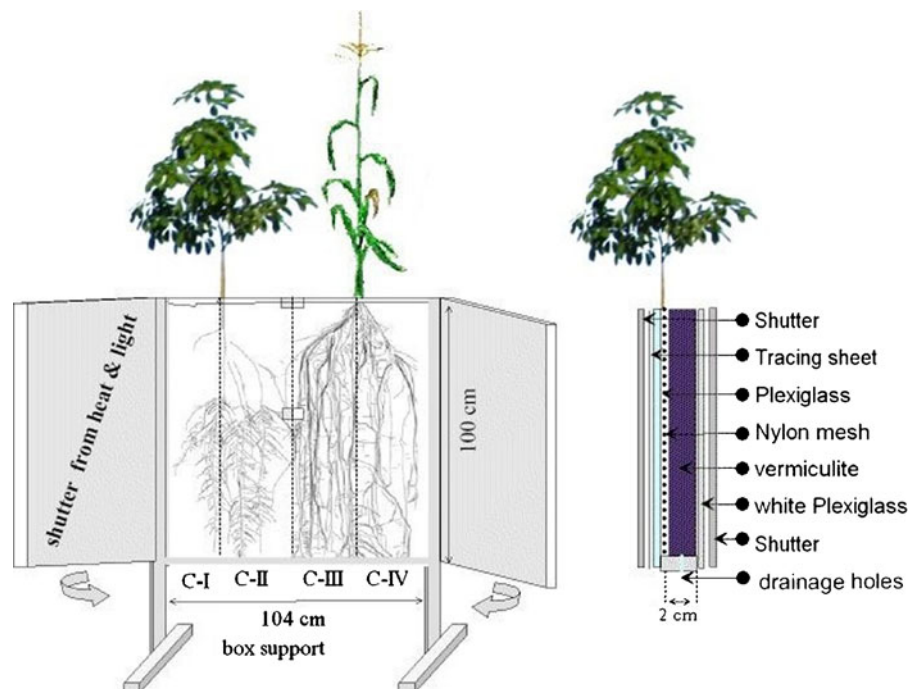
Rhizoboxes

The rhizoboxes were containers 2 cm thick 100 cm high and 104 cm wide (internal dimensions). The back of each rhizobox was made of 4 mm thick, opaque PVC. The sides and bottom were made of 2×2 cm square aluminum. Drainage holes (2 mm in diameter) were perforated at 5 cm intervals through the bottom of the rhizobox. The front plate was made of 4 mm thick transparent Plexiglas (Pan Asia Industrial Co., Ltd.). As investigated elsewhere, acrylic plastic shows no detrimental effect on the growth and survival rate of roots (Withington et al. 2003.) A transparent acetate sheet (Polyplex Plc., Ltd., Thailand) was placed over the Plexiglas in order to record root development at regular time intervals (Fig. 1). To minimize photo- and thermo-tropic responses from the roots, shutters lined with black plastic and thick aluminium foil were attached to the front of the rhizoboxes at all times, except when roots were traced on acetate sheet. The transparent wall was exposed only for the purpose of root tracing.

Each rhizobox was packed with moist vermiculite (maximum grain diameter of 2 mm, Agra-vermiculite, Dutch Greenery Co., Ltd.). A nylon mesh (average pore diameter: 150 µm) was stretched over the vermiculite, behind the front Plexiglas pane. Most of the root system (on average 87% and ~100% of the dry biomass of corn and rubber tree, respectively) was thus constrained to develop in the virtually two-dimensional space between the transparent front pane and the nylon mesh. Roots that did not grow within this thin gap, grew behind the nylon mesh, directly into the vermiculite.

Rubber tree seedlings were implanted between the window and the nylon mesh. It was necessary to create a deep and wide enough groove in the vermiculite to insert the rubber tree root systems in the thin rhizoboxes. Plants were transplanted with some vermiculite still attached to the root system. No pruning was conducted on the root systems at this stage. Rubber trees and corn plants were implanted in identical positions, 50 cm apart from each other, in all replicate rhizoboxes. The plants were kept under non-limiting supply of water and nutriments via daily watering with Hoagland's solution (Epstein and Bloom 2005); the solution was supplied by capillarity using a thick wick in contact with the whole surface of the vermiculite, at the top of the box. Supply was stopped once some solution started to drain freely from the bottom of the rhizoboxes.

Environmental conditions

The experiment was carried out under an open-sided shelter covered with a translucent plastic roof that transmitted approximately 56% of the incident light. Air temperature, relative humidity and the photo-period were recorded daily over the whole duration of the experiment. The average air temperature, relative humidity, photo-period and photosynthetically active radiation were 29.2°C, 81.6%, 8.4 h and 410 µmol $m^{-2} sec^{-1}$, respectively (from observations over the 77-day period of the experiment).

Measurements

Digital representations of root systems using the DART software

Root branching and elongation of both rubber tree and corn were monitored every second day until corn ears were fully formed. This was achieved by tracing, at each observation date, the newly grown root segments on the transparent plastic sheet placed over the front pane, using waterproof colour pens, each colour being specific of the date at which the root segments were observed. At the end of the experiment, the transparent sheets were scanned using an A4 Epson Perfection V700 Photo, at resolution of 600 dpi: the whole surface area of each 50×100 cm transparent sheet was scanned as 12 separate A4 sections which were subsequently stitched together using the GNU Image Manipulation Program (Gimp 2.6—2009, http://www.gimp.org/) to produce a mosaic image of the tracings corresponding to the entire root system. Using these images, all root systems were finally digitized using the DART software (Le Bot et al. 2009; http://www.avignon.inra.fr/psh/outils/dart_software), which produces a description of root system architecture as a series of ordered links to which is attached a list of specific attributes, including: an identification number, the branching order, the date of emergence, the parent root identification number, the distance to the current's and parent's root base, and the cumulative root length at each observation date. DART is

particularly suitable for developmental analyses of complex root system architectures.

Analysis of root system dynamics at the scale of the entire root system

Since they include chronological information about the elongation of individual roots, DART output files were particularly useful to compute parameters related to root system dynamics, such as the overall root length, root length density and growth rates, at any given time.

In particular, root system dynamics was investigated via the computation, over four time intervals, namely 0–10, 10–15, 15–20 and 20–30 DAS, of daily changes in root length density (RLD, in cm cm^{-2} day^{-1}) in four 25 cm wide and 100 cm high vertical compartments, consisting of two inner compartments, C-II, on the rubber side and C-III on the corn side, enclosed in between the outer rubber (C-I) and corn compartments (C-IV) (Fig. 1). This was achieved, for every time interval, by dividing the total root length included in a given vertical compartment, by the surface area of the compartment and the time interval's duration.

Root system dynamics was also investigated using the 'root system daily expansion', which, for a given root system, corresponds to the overall root length increase over a period of 24 h, and is expressed in cm day^{-1}. While this parameter is equivalent to the number of actively growing root apices multiplied by an average root growth rate, here, it was derived from the DART records as the difference in root system overall length between two observations, divided by the time lag between two observations (2 days).

Finally, we computed the total number of roots included in root systems as a function of time, as a simple indicator of root branching activity.

Root system trajectories

DART outputs were also used to assess the displacement and the trajectory of entire root systems. In this respect, root system growth was considered a diffusion-type process (De Willigen et al. 2002) that proceeds in the downward direction as a function of individual root branching and elongation. Within this conceptual framework, the elongation of every individual root at a given time step is seen as a vector that describes the local movement of the diffusion front; the overall expansion of the root system was hence estimated by computing the vectorial sum of all the individual vectors describing root elongation. Finally, the trajectory of root growth expansion was represented graphically as the chronological accumulation of the vectorial sums calculated at each time step. Based on this approach, a balanced root system development with gravitropic main axes and as many branch roots of similar length on both sides of these main axes, should result in a vertical, downward trajectory. In contrast, any preferential allocation of assimilates to support the growth of main axes or branches in a particular direction should result in a deviation of the root system trajectory in the same direction.

Root growth patterns at the individual root scale: root encounters

Using the DART software, root encounters were visually identified in the digitized root systems of the transparency sheets. Variations in root elongation rates during root encounters were estimated using the link coordinates and temporal information, as recorded in DART output files, of at least three root segments of a given root, corresponding to pre-, syn- and post-contact conditions.

Two types of root encounters can occur, namely "crossings" which correspond to the growth of one root towards another root, then around and beyond it, and "parallel contacts" which correspond to one root growing towards another root and then changing its growth direction so that it subsequently grows parallel and in close contact with the other root. These two types of root contacts can occur within the root system of an individual plant (intra-individual root contact) or involve roots of two neighbour plants (inter-individual root contact). Given the design of our experiments, all inter-individual contacts were necessarily inter-specific.

Most roots exhibit a determinate growth pattern, particularly the finest (Cahn et al. 1989). However, due to plasticity, root growth patterns are not as strictly defined as that of fruits and leaves (Pagès 2000). Declining elongation with root age has been reported for corn axile roots (Pellerin and Pagès 1994). Finite root growth patterns have also been described for rubber tree (Le Roux 1994). In addition, root develop-

ment in young rubber seedling is periodic and related to leaf expansion, indicating within-plant competition for assimilates (Thaler and Pagès 1996). Therefore, it cannot be excluded a priori that a change in elongation rates (particularly a reduction), concomitant with a root encounter, might not or only partly, be a consequence of the encounter. To circumvent this potential confounding effect, we compared pre-, syn- and post- encounter root growth rates with growth rates of other roots of the same age classes (in days after sowing, DAS).

The time of contact was defined as the latest observation period over which the contact point was reached by one of the two roots. This avoided generating apparent inconsistencies between the average times of pre-, syn-, post-contact observations for corn and rubber, since many rubber tree roots were contacted by corn apices far behind their own apices.

Additional data and statistical analysis

Basic parameters related to the growth of the shoot were also collected during the experiments, including leaf area and stem length. Data exploration, statistical analysis, and graphical representations of all the data collected during the experiments described in this paper were performed using the R language environment for statistical computing and graphics, version 2.9.1 (R Development Core Team 2009). The two-tailed unpaired (independent) Welch t-test was used to compare parameters for which replicate samples could be obtained (identically distributed or not); such variables include overall root length, root system daily expansion rate, stem length, and leaf area. The two-tailed paired (dependent) two-sample Welch t-test was used to compare parameters for which independent samples could not be obtained, such as, typically, growth rates of individual roots at different times with respect to a root encounter. The Welch t-test was systematically used as in most cases the assumption of equal variances between samples could not be verified.

Results

General characteristics of the rubber tree and corn root systems

All rubber tree plants had a developed root system at the onset, including a pruned taproot (first order) and branches (secondary to fourth order). In all replicate experiments, either with or without corn, the growth of the pruned taproot was relayed by lateral roots through the process of reiteration (Oldeman 1974; Atger and Edelin 1994). This permitted vertical, downward exploration of the growth medium by rubber tree root systems.

By the time observations were discontinued (77 DAS), ~2.5%, 43% and 54.5% of the total number of corn roots were axile, 1st order laterals and other lateral roots, respectively. In rubber trees, 5%, 38% and 57% of the total number roots were secondary branches of the acropetal sequence, 2nd order laterals (or tertiary roots) and 3rd order lateral roots. In corn, the length distribution of lateral roots was asymmetrical, for all phytomers (mean: 15 mm; median: 9.6 mm). Due to differences in plant age and transplantation, the root classification established by Le Roux and Pagès (2000) on illegitimate GT1 clones could not be applied straightforwardly to the rubber trees analyzed here.

Root growth patterns at the scale of the entire root system

Despite 1–2 day shifts between individuals, all three corn plants exhibited a series of four to five10-day growth cycles, from 11–15 DAS until 49–57 DAS (Fig. 2) according to which the daily root system expansion varied. This period (11–15 to 49–57 DAS) is also the period of most active corn root system expansion, with rates exceeding 75 cm day^{-1} and reaching up to almost 525 cm day^{-1} (Fig. 2). The precise timing and the amplitude of these cycles varied depending on the replicate and were not related to variations in environmental conditions (air temperature, PAR, or photo-period).

In addition, rubber and corn root expansion rates tended to vary concomitantly; although the frequency of our observation (every second day) was not sufficient to resolve a putative correlation between the growth rates of the two associated plants, in 70% of all observations periods, positive and negative variations in root growth rate occurred within the same 2-day period in the two plant species.

In corn, root branching followed three main phases (Fig. 3): from 0 to 17 DAS, during which root numbers increased little, indicating limited branching; from 17 to 50 DAS, during which branching

Fig. 2 Root system daily rates of expansion (in cm day^{-1}, on a logarithmic scale) for the three replicate treatments corn x rubber tree. Note the series of approximately 10-day growth cycles, from 11–15 to 49–57 DAS, during each of which, the daily expansion of both corn and rubber tree root systems oscillated concomitantly
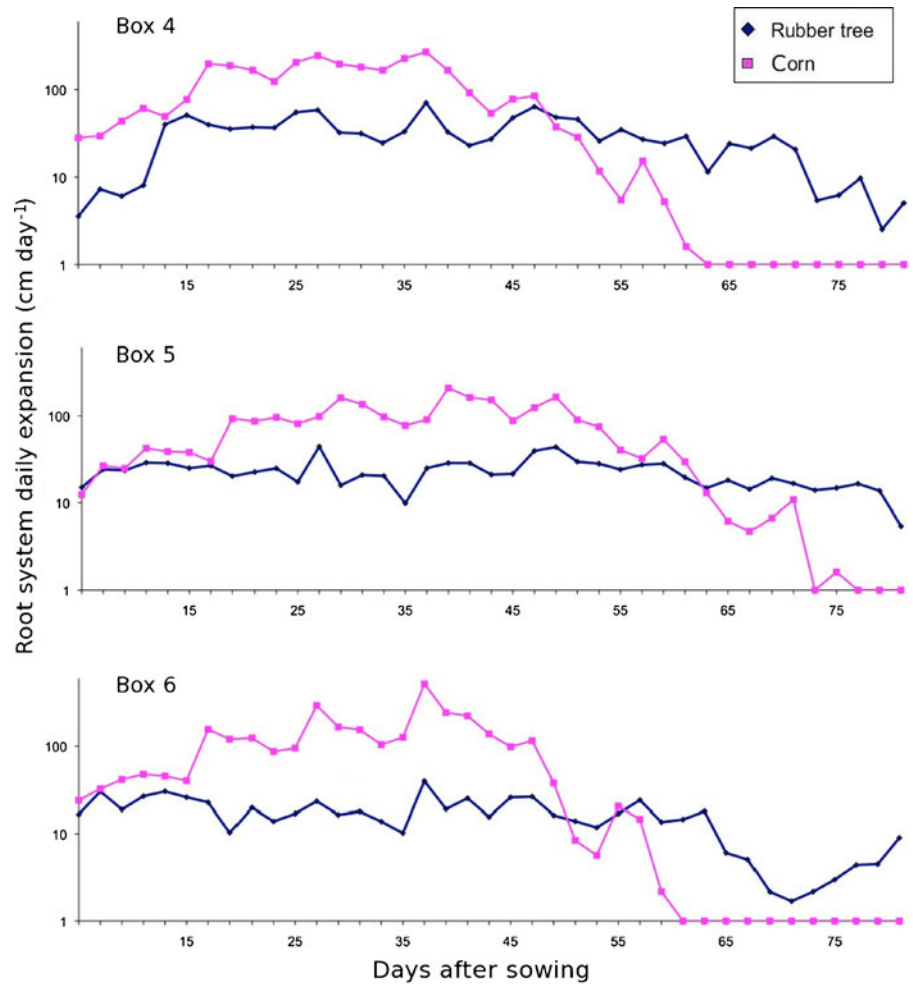
produced about 20 more roots every day; and beyond 50 DAS, corresponding to the end of the branching period with no or little further increase in root numbers. From these observations we can therefore define the 17–50 DAS period as the period of active corn branching.

In rubber, root branching followed the first two phases described for corn but the reduction in root branching from 50 DAS onward did not apply (Fig. 3): in one case only (Box 6) the branching rate clearly dropped after 61 DAS, but in the two other cases, there was only a slight and progressive reduction of branching rate after 50 DAS compared to that of the 17 to 50 DAS period. In addition, one plant (Box 4) had a much higher branching rate than the two others.

Overall root length, stem length and leaf area are reported in Fig. 4a. Root system daily expansion rate is reported in Fig. 4b. The root systems of control rubber trees expanded at significantly slower growth rates (14 cm day^{-1}) than rubber trees grown in association with corn (53 cm day^{-1}) (Fig. 4b; $p<0.001$ Welch Two Sample t-test). Remarkably, this increase in rubber tree root growth rate occurred only during the period of active corn root system expansion: as soon as corn root systems expansion slowed down, i.e. from 49–57 DAS onwards, the average expansion of rubber tree root systems also dropped to about half its previous value (23 cm day^{-1}) which is still significantly higher ($p<0.05$ Welch Two Sample t-test, $n=9$) than the average expansion of the three control rubber trees.

Despite these differences related to root system expansion, both control rubber trees and those associated with corn developed similar overall stem length, leaf area, and total root length: even if rubber trees grown in association with corn grew almost
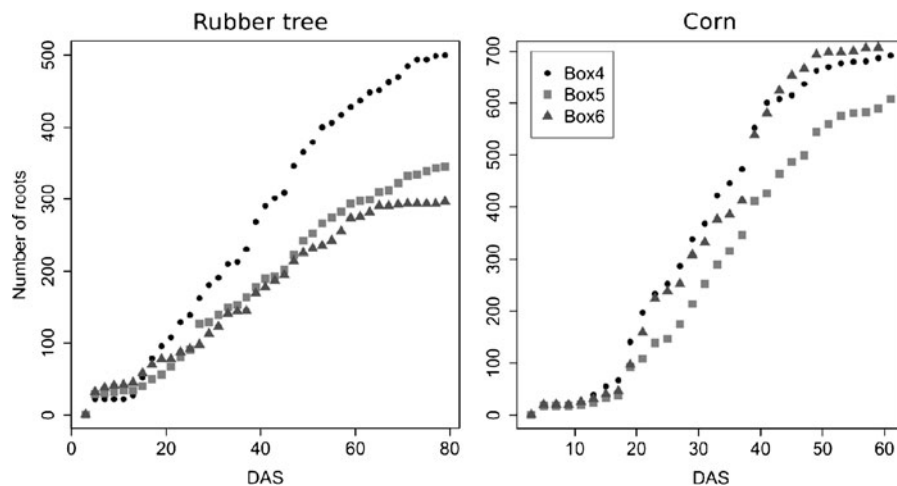
Fig. 3 Cumulative distribution of root numbers as a function of time, left: rubber tree; right: corn. Root branching intensity corresponds to the slope of the distributions. In corn, three main phases, 0–17, 17–50 and more than 50 DAS can be identified. The period of most active corn branching spans from 17–50 DAS. In rubber, root branching followed the first two phases described for corn but the reduction in root branching from 50 DAS onward did not apply. One plant (Box 4) had a much higher branching rate than the two others

twice as much root length, on average, as that of controls, this could not be confirmed as a significant difference at the tree level, probably because of the limited number of replicate root systems available (n=3). The fact that rubber trees grown in association with corn grew about three times faster and developed almost twice as much root length as controls also indicates that only some of the roots grew faster (a maximum of about 66%, assuming that the remaining 33% stopped growing altogether).

All three corn plants grown in association with rubber trees systematically increased RLD faster in compartment C-III (Fig. 5), followed by compartments C-IV and C-II. Over the 0–30 DAS period represented in Fig. 5, RLD increase reached a maximum, between 10 and 15 DAS in two cases and between 15 and 20 days in one case. In two out of three replicates, corn developed substantial amounts of roots in compartment C-I (Fig. 5). On the other hand, two out of three rubber trees increased RLD faster in compartment C-I, followed by compartment C-II. For rubber trees, RLD variation was the highest between 20 and 30 DAS, i.e. after corn had reached its maximum growth rate. Noticeably, the replicate rhizobox in which the rubber tree increased RLD the fastest in compartment C-II, was also the one where the corn increased RLD at the lowest rate in this same compartment (Fig. 5).

Finally, it must be noted that from the first days of the growing period over which this study was conducted, corn grew unusually long laterals (of the order of 20–30 cm). These laterals were emitted from axes located on the side of the corn root system that was closest from the rubber tree (Fig. 8) and contacted the rubber tree root system as early as 10 to 15 DAS.

Root system trajectories

Although the root system trajectories of the three corn plants differed with regards to the values of the vertical and horizontal components and the precise timing of change in growth pattern, all had common characteristics (Fig. 6), namely: an initial phase of about 12–15 days during which there consistently was a preferential growth in the direction of the rubber tree, a second phase, from c. day 15 to c. day 40, during which the opposite trend prevailed, and a final phase during which root system development was generally more balanced (Fig. 6). However, the corn plant in box 4 was the closest to a balanced root system trajectory overall, while that in box 5 was the most laterally spread out, resulting in a shorter cumulative vertical component, and that in box 6 strongly shifted to the direction opposite to the rubber tree from c. day 35 to c. day 45.

Trajectories of rubber trees associated with corn were less variable, with more gentle lateral shifts than that observed for corn, and smaller horizontal and vertical cumulative components than corn. Compared to rubber
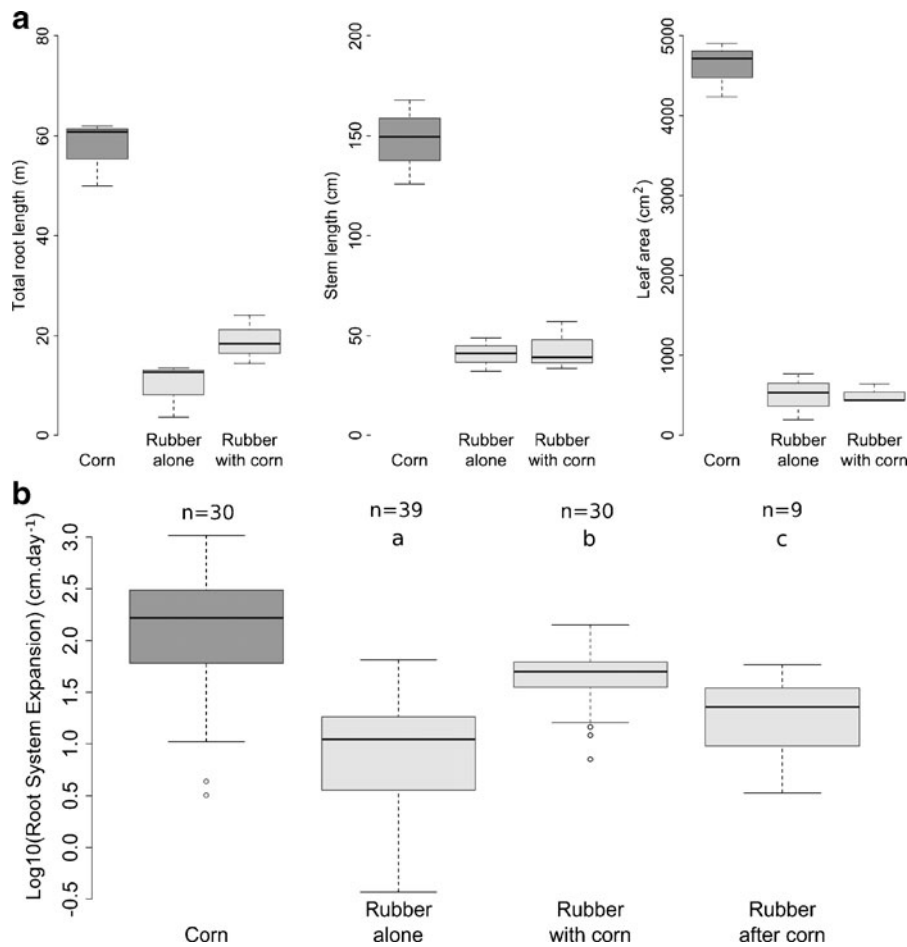
Fig. 4 a Box-whisker plots of total root length (*left*), stem length (*center*) and leaf area (*right*) at the end of the experiment (77 DAS). All corn parameters are significantly different from that for rubber tree ($p < 0.001$ Welch Two Sample *t*-test, $n=3$). The central horizontal line indicates the median value, and the upper and lower edges of the box (hinges) indicate the 25th and 75th percentile values, while the whiskers extend 1.5× the spread of the hinges. Data points outside this range are indicated with circles. **b** Box-whisker plots of root system daily expansion rates, expressed as the decimal logarithm of the actual values to improve visualization. Root system daily expansion rates of control rubber trees (0–77 DAS), rubber trees with corn during active corn root system expansion (15–49 DAS), and rubber trees after active corn root system expansion (49–77 DAS) all differed significantly from each other ($p < 0.05$ Welch Two Sample *t*-test, n indicated on top of each box-whisker) as indicated by the a, b and c letters, respectively, above the box-whisker plots

trees grown with corn, rubber trees alone had trajectories with a much shorter cumulated vertical component (Fig. 7), which indicates that the main effect of growing rubber in association with corn was to stimulate downward growth, but not really to reorient the overall growth of rubber tree root systems.

Even though there was no simple relationship between the trajectories of corn and associated rubber, the end of the initial phase of corn root system expansion towards the rubber tree coincided, in all three replicates, with the time at which corn roots first encountered rubber tree roots: in Fig. 6, which shows root system trajectories, each arrow represent a time lag of two days, and in all three replicates, corn trajectories changed direction roughly 12 DAS, which is also roughly the time of the first root encounters as circled in the second panels from the left hand side in Fig. 8.

Root contacts

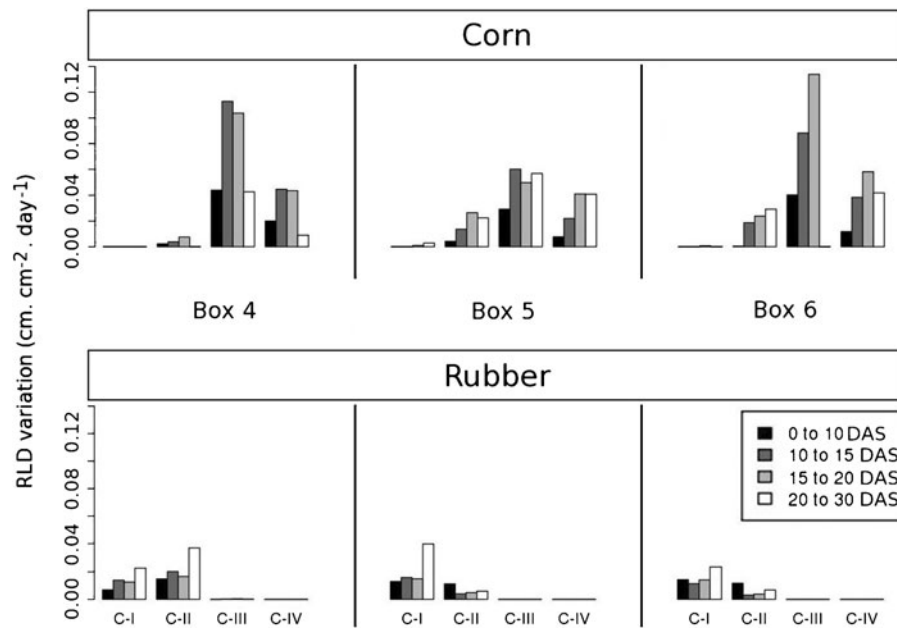Overall, out of the three replicate corn×rubber experiments, we could identify 90 inter-specific

Fig. 5 Changes in Root Length Density (RLD) for corn (*top row*) and rubber tree (*bottom row*) grown in association, over three successive periods, namely 0–10, 10–15, 15–20 and 20–30 DAS, in the four adjacent compartments (25-cm wide and 100-cm high). C-I: rubber tree side, edge of the box; C-II: rubber tree side, centre of the box; C-III corn side, centre of the box; C-IV: corn side, edge of the box. In rubber, in two cases root growth rates were higher on the side opposite to corn (C-I) while in corn root growth rates were higher in the compartment next to the rubber tree (C-III)

contact points out of which 12% were parallel contacts. In all cases, rubber tree roots were contacted by corn roots, and a single corn root could encounter up to 17 rubber tree roots. Because of the low occurrence of parallel contacts, and because they are likely to be induced by experimental conditions (the space left for growth in the third dimension was extremely limited) we did not analyze them as a specific case.

Inter-specific root contacts

Inter-specific root contacts occurred as early as 10 and as late as 74 DAS. On average, corn roots involved in root contacts were 2nd and 3rd order laterals that had an elongation period of over 19 days (minimum: 12, maximum 32). In rubber tree, roots involved in contacts where 1st to 3rd order laterals with an average elongation period of 25 days (minimum: 10, maximum 42). Pre- and post-contact growth rates of rubber tree roots that encountered corn roots, significantly slowed down from 0.73 to 0.21 cm day^{-1} ($p<0.001$; $n=32$ Welch Two Sample t-test; Table 1) while that of corn roots that encountered rubber tree roots

decreased from 2.21 to 1.11 cm day^{-1} ($p<0.001$; $n=33$ Welch Two Sample t-test; Table 1). By comparison, the growth rates of rubber tree and corn roots sampled over time intervals that encompassed the average times of pre-, syn-, and post- root encounters did not significantly vary (Table 1). Therefore, the time-related decline in elongation of corn axile roots reported by Pellerin and Pagès (1994) does not appear to be a likely confounding factor that would explain the change in elongation observed for corn roots that encountered rubber tree roots. These results indicate that inter-specific root encounters induced a significant reduction in both rubber tree and corn root elongation rates.

Intra-specific root contacts

There was a significant ($p<0.001$) decrease in rubber tree root elongation rate following encounters with another rubber root (Table 2). In contrast, intra-specific encounters did not alter the elongation rates of corn roots. Intra-specific contacts occurred about 10 days earlier on average in corn than rubber (Table 2).

Fig. 6 Root system trajectories of the three corn plants (*top row*) and associated rubber trees (*bottom row*). Trajectories are composed of a succession of arrows representing the vectorial sum of all root growth over a two-day observation period. Successive two-day periods are indicated by different colours
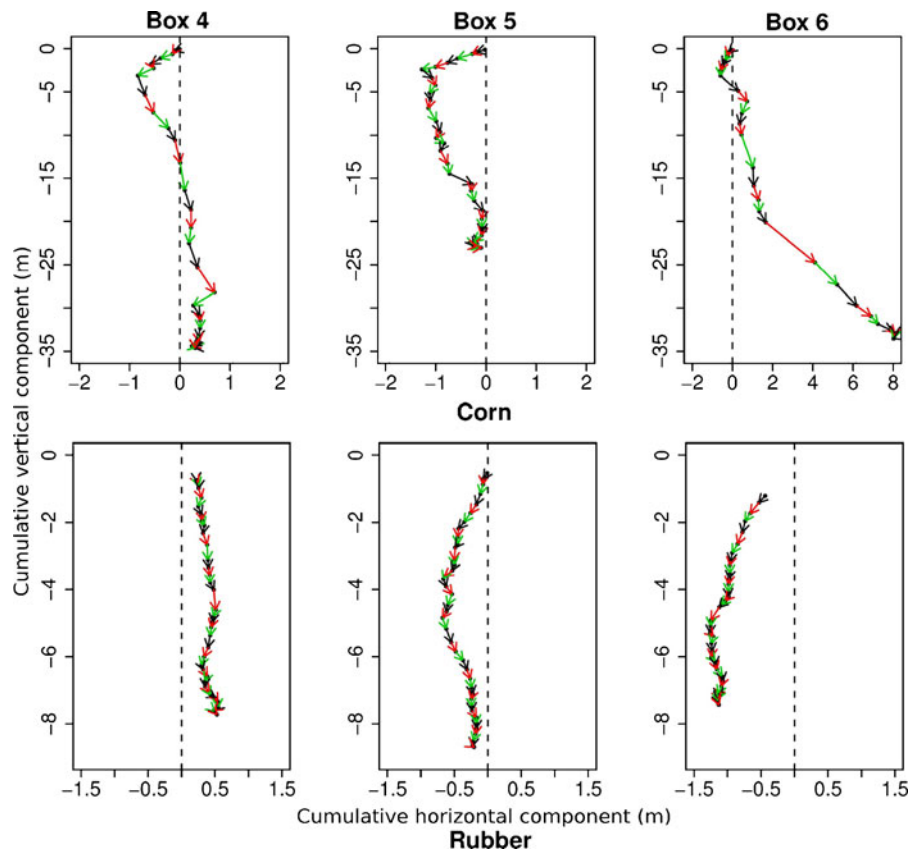

Discussion

This study investigated whether and how an annual (corn) and a perennial (rubber tree) grown in association affect each other's root system placement and growth dynamics. While there exists a vast body of literature on below-ground interactions between plants, most of the existing research on this topic is based either on static observations of root system geometry (Schenk et al. 1999), or on the analysis of

provoked roots encounters (Mahall and Callaway 1991). In contrast, this study examines the dynamics of below-ground interactions in an inter-specific system, at both the whole root system level and that of the individual root, based on detailed digital descriptions of root systems generated using the DART software (Le Bot et al. 2009).

Based on this approach we could show that corn tended to have a 'territorial' behaviour, colonizing preferentially the space towards rubber trees, until

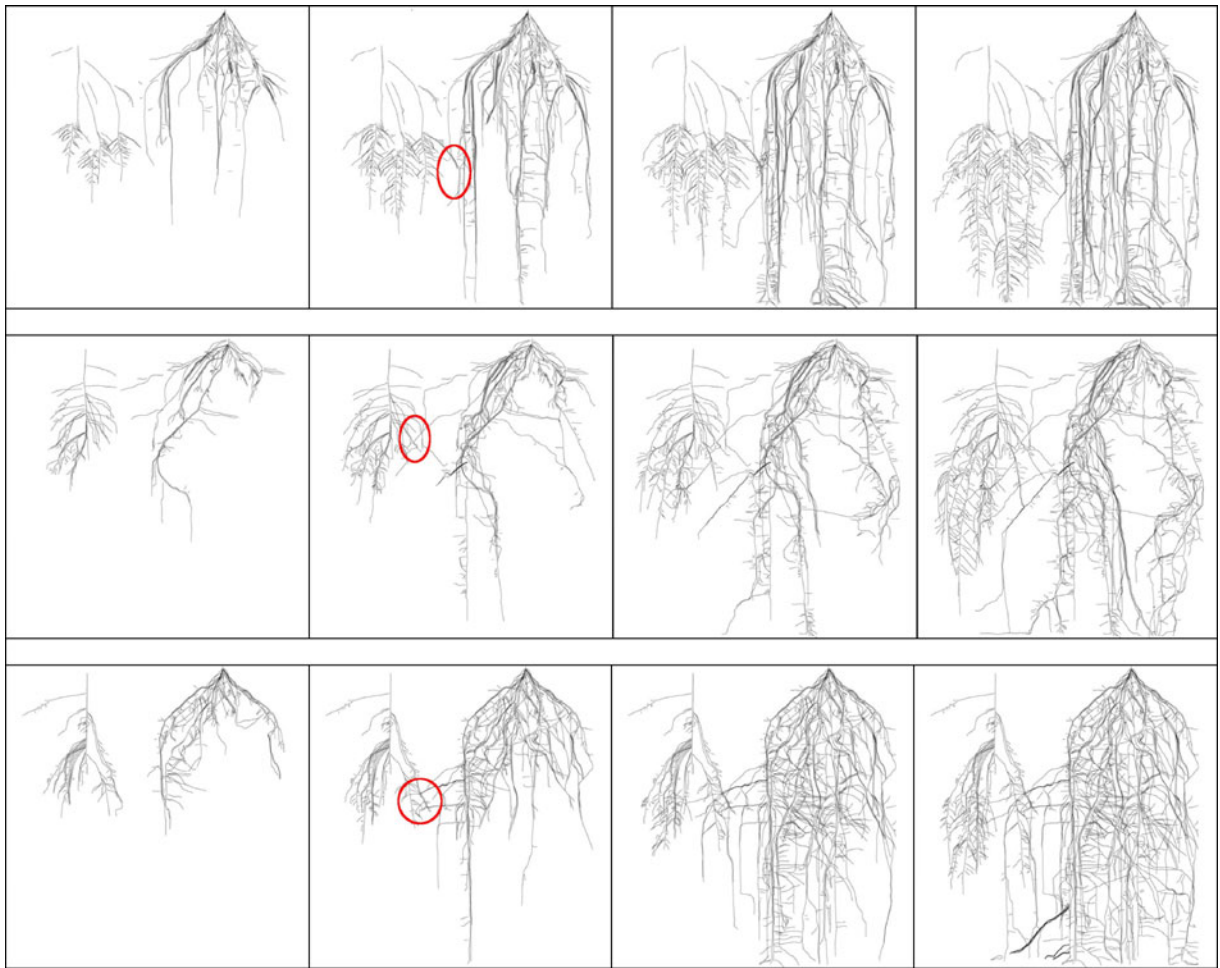**Fig. 7** Root system trajectories of the three control rubber trees. See Fig. 6 for detail
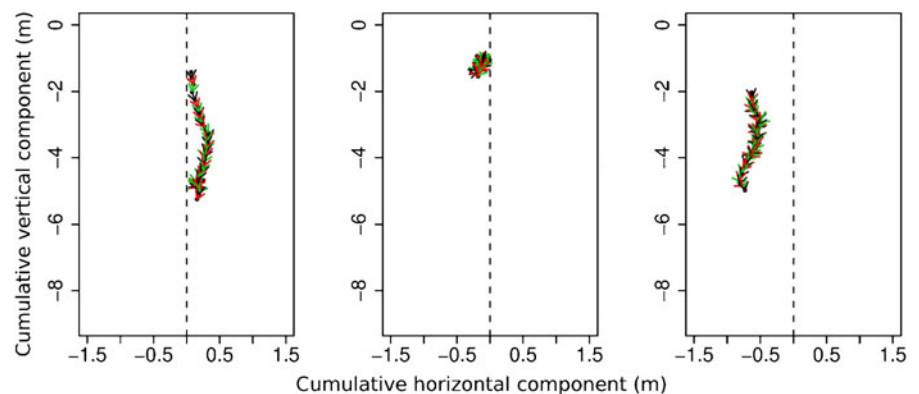
Fig. 8 Root system architecture of the corn and rubber tree plants in the three replicate rhizoboxes over four successive periods, namely 0–10, 10–15, 15–20 and 20–30 DAS (from *left* to *right*). In all cases, corn extended long lateral branches that encountered the adjacent rubber tree root system during the second period, as indicated by circles overlaid on the corresponding images

encounter; we also found a positive coordination between rubber and corn root systems expansion. To the best of our knowledge, this is also the first time that growth cycles are reported for corn root system expansion, most likely because previous studies of corn root systems using rhizoboxes have been carried out over shorter periods, without the quantifying power of a tool such as DART. These growth cycles have a roughly 10-day periodicity over the period 11–15 to 49–57 DAS and are most likely in phase with corn phenology.

Digitizing complex root architectures such as that studied in this work was labour intensive: root systems included, on average, more than 4,000 individual links, which required about 40 h of work per root system. The limited number of replicates dealt with in this study is a direct consequence of the labour-intensive nature of this digitizing work. However, this limitation was counterbalanced by the fact that, once digitized, a vast range of root growth indicators could be computed. Here, we demonstrate that novel approaches such as the study of root system growth trajectories provided new insights into below-ground plant interactions, this being based on DART records.

The duration of the experiments was long enough for root system development to result in spontaneous inter-specific root encounters. In contrast with previous studies (e.g. Mahall and Callaway 1991), no particular measure was taken to provoke root encounters,

Table 1 Statistical summary of inter-specific root encounters. P-values correspond to paired two-sample Welch t-tests (two-tailed). (a)–(b), (a)–(c) and (b)–(c) refer to the differences between the mean pre- and syn-, pre- and post-, and syn- and post-encounter growth rates, respectively. Pre-, syn- and post-encounter root growth rates were compared with growth rates of other roots of the same age classes to avoid computing statistics that include the potential confounding effect of changes in elongation rates with time. The age classes of these roots are reported in brackets as '*Sampling date range*'; the figure to the right of the closed bracket and to the left of the open bracket indicating the lower (inclusive) and higher (exclusive) limits of the sampling date range, in DAS

Inter-specific root encounters			
	(a) Before	(b) During	(c) After
Average observation time (DAS)	27	37	43
Corn Roots			
Mean growth rate (cm day^{-1})	2.21	1.56	1.11
p-value (n= 33)	(a)–(b): 0.014	(a)–(c): <0.001	(b)–(c): 0.049
Rubber Roots			
Mean growth rate (cm day^{-1})	0.73	0.62	0.21
p-value (n= 32)	(a)–(b): 0.256	(a)–(c): <0.001	(b)–(c): <0.001
Comparison with all other Roots			
	(a) Before	(b) During	(c) After
Sampling date range (DAS)	[24–30[	[34–40[	[40–46[
Corn Roots			
Mean growth rate (cm day^{-1})	1.86	1.90	2.21
p-value (*n*=105)	(a)–(b): 0.479	(a)–(c): 0.314	(b)–(c): 0.357
Rubber Roots			
Mean growth rate (cm day^{-1})	0.68	0.86	0.83
p-value (*n*=117)	(a)–(b): 0.243	(a)–(c): 0.337	(b)–(c): 0.453

although by design, rhizoboxes induced a virtually 2–dimensional root system development, which may be seen as factor maximizing the likelihood of inter-individual root contacts. Nevertheless, the spacing between plants (50 cm), was of the same order of magnitude as common inter-row spacings implemented in the field.

The growth and architectural characteristics of root systems analyzed in this work, particularly the overall root lengths, proportions of different branching orders, and root elongation rates were consistent with values of previous reports; this indicates that, despite the limited number of replicates, our experimental conditions did not introduce any bias likely to invalidate the results presented. For example, consistent with previous reports (Pagès and Pellerin 1994), the length distribution of corn lateral roots was of the same order of magnitude and highly asymmetrical, for all phytomers (mean: 32 mm; median: 10 mm). Similarly, growth rates of both corn (Pellerin and Pagès 1994) and rubber tree roots (Le Roux 1994) were comparable to that previously reported.

The results of this study suggest that plants grown in association in rhizoboxes were able to sense and adjust their root system development according to that of their neighbour. Specifically, such a scenario is supported by the fact that: 1. the growth trajectories of corn root systems were initially oriented towards the rubber trees, 2. corn plants grew unusually long laterals when some of their main axile roots were close to rubber tree roots, 3. both individual corn and rubber tree roots grew at lower elongation rates following encounters with each other, 4. the overall root length expansion of rubber trees was significantly higher in the presence of a corn neighbour and while the overall growth rate of corn was the highest (i.e. until ear formation), and 5. rubber and corn root expansion rates varied concomitantly. Due to the absence of the corn alone treatment, we cannot conclude firmly about the influence of a rubber tree

Table 2 Statistical summary of intra-specific root encounters. See Table 1 caption for meaning of comparisons, p-values, and sampling date range. Note that 'During' and 'After' values for comparisons with other corn and rubber roots had to be pooled together due to the fact that a sampling date range long enough to allow the computation of a growth rate could not be defined otherwise

Intra-specific root encounters			
	(a) Before	(b) During	(c) After
Corn Roots			
Average observation time (DAS)	22	24	27
Mean growth rate (cm day^{-1})	2.11	2.54	2.21
p-value (*n*=54)	(a)–(b): 0.171	(a)–(c): 0.733	(b)–(c): 0.245
Rubber Roots			
Average observation time (DAS)	26	35	35
Mean growth rate (cm day^{-1})	0.75	0.71	0.46
p-value (*n*=68)	(a)–(b): 0.564	(a)–(c): <0.001	(b)–(c): <0.001

Comparison with all other Roots		
	(a) Before	(b) During and After
Corn Roots		
Sampling date range (DAS)	[20–24[	[24–30[
Mean growth rate (cm day^{-1})	1.73	1.86
p-value (*n*=86)	(a)–(b): 0.449	–
Rubber Roots		
Sampling date range (DAS)	[24–28[	[34–38[
Mean growth rate (cm day^{-1})	1.00	0.87
p-value (*n*=102)	(a)–(b): 0.417	–

neighbour on the growth trajectory of corn, even though this is somewhat supported by the observation that corn plants grew unusually long laterals towards their rubber neighbours. Since care was taken to supply water and nutrients in unlimited amounts, it is difficult to invoke competition for resources to explain the observed rooting patterns.

However, while we observed that corn roots developed towards rubber roots until a contact was established, it is not possible to dismiss the fact that the initial corn root system growth towards rubber tree roots could have been related to the original orientation of the radicule growth, particularly given the fact that the radicule's geotropism is rather weak. In addition, a deviation of the trajectory from the vertical becomes all the more significant that it results from the contribution of a large number of roots, but in all three corn replicates, the most intense branching started only 20 DAS i.e. a period for which corn root system trajectories were already either vertical or, in one case, oriented in the direction opposite to rubber.

In the case of rubber trees, changes in root system trajectories were of much lower magnitude than in corn, and it therefore remains unclear whether the two studied species are able to deploy the same strategies to adapt their root system development to that of their neighbour. The effect of the location and orientation of the laterals that relayed the taproot was likely influential in the development of the trajectories of rubber tree root systems. In addition, the lack of space for rubber roots to further expand where corn roots had already grown is yet another possible explanation of the root patterns and trajectories observed in rubber trees: there was very limited space available for roots to pass each other in between the nylon mesh and the front pane, and rubber roots being on average thicker than corn roots, they might have been physically stopped by corn roots. It is also possible that, given the differences in average root system expansion between the two plants (corn producing at least 5 times more root length daily than rubber trees), rubber trees could not pre-emptively outcompete corn. While

self-inhibition, i.e. reduced resource allocation to less promising parts of the root system (Falik et al. 2003, 2005) appears to have possibly been at play in rubber trees, which displayed reduced root elongation in both inter- and intra-specific root encounters, this mechanism cannot be invoked for corn which did not react to intra-specific encounters.

The literature suggests that roots are able to detect and avoid the presence of neighbouring roots (Krannitz & Caldwell 1995), and to segregate spatially in 'territories' (Schenk et al. 1999). At the whole root system level, root segregation can provide competitive advantages for water and nutrient uptake (Casper and Jackson 1997) and limit overlap between individual root systems (Brisson and Reynolds 1994). Segregated root placement could, at least partly, result from an avoidance mechanism of soil volumes under the influence of other plants, such as soil containing exudates of other roots (Krannitz and Caldwell 1995). Falik et al. (2003) attributed the absence of inter-individual root contacts in *Pisum sativum* to self/non-self discrimination. However, such results do not preclude the possibility that, in other species, self/non-self discrimination could promote root growth towards a neighbour and even root encounters. It has been argued that chemically based allo-recognition is unlikely due to rapid decomposition of organic compounds used as 'identifying molecules' (Falik et al. 2003). Alternative mechanisms have been suggested, such as a combination of hormonal and electrical oscillations (Souda et al. 1990) that might be perceived by neighbouring roots without direct contact.

Some of the findings of this study appear worth being investigated further, from an agronomic perspective. For example, over the short observation period of our experiment, neither the above- and below-ground parts of rubber trees were negatively affected by the presence of nearby corn. If this were confirmed under a wide range of field conditions and over longer periods of time, this would be of influential importance for farming practices. Timing issues need to be documented in detail. For example, Collet et al. (2006) reported that the size of oak root system was considerably reduced by grass competition. It therefore seems important to clarify the roles of seasonal inter-crops from that of perennial covers.

Similarly, it appears worth investigating further whether and how the 'territorial' rooting behaviours observed in rhizoboxes occur in the field, and how

they could be used to shape the root system architecture of rubber trees. Ecological research on underground interactions between plants has recently indicated that competition for bio-available nutrients is driven by diverse mechanisms and strongly depends on soil, nutrient, and plant properties (e.g. Raynaud et al. 2008): facilitation and competition would dominate under high- and low-stress conditions, respectively. Accordingly, on heavily weathered, phosphorus deficient soils, the acidification of the rhizosphere by faba bean inter-cropped with corn was found to mobilize phosporus in soil volumes and amounts sufficient to benefit the growth and yield of corn (Li et al. 2007). Similarly, using facilitating, inter-crop based techniques to stimulate rubber tree root growth towards deeper and moister soil layers could prove beneficial for the long-term productivity of a plantation. This could be of particular interest in locations where a seasonal drought prevails. A final area of needed research is to understand how root growth coordination translates in terms of crop yields. It has been reported that spatial and temporal shifts in rooting patterns induced by inter-crops can be detrimental to the yields of one of the species, but this is attributable to competition between root systems for one or more resources (Celette et al. 2005; Collet et al. 2006; Li et al. 2006), a process that was eliminated in our simplified experimental setup.

However, and more unexpectedly, other reports show that combining plant species can lead to yield increases, putatively due to enhanced soil exploration (Li et al. 2006; Mulia and Dupraz 2006; Malezieux et al. 2009). While detailed analyses of intra- and inter-specific root interactions such as that presented in this paper are not sufficient to fully unravel how species grown in combination can functionally complement each other, they represent an essential step towards the design of sustainable agro-ecosystems, which are much needed to meet the worldwide growing demand for food.

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