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Research article

Assembly history modulates vertical root distribution in a grassland experiment

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The order of arrival of plant species during assembly can affect the structure and functioning of grassland communities. These so-called priority effects have been extensively studied aboveground, but we still do not know how they affect the vertical distribution of roots in the soil and the rooting depth of plant communities.

To test this hypothesis, we manipulated the order of arrival of three plant functional groups (forbs, grasses and legumes) in a rhizobox experiment. Priority effects were created by sowing one functional group 10 days before the other two. Rhizoboxes in which all functional groups were sown simultaneously were used as controls. During the experiment, the total visible root length and the mean and maximum rooting depth of plant communities were monitored using image analysis and a new methodological approach using deep learning (RootPainter) for root segmentation. At harvest, we measured aboveground (community and species level) and belowground (community level) biomass, and assessed the vertical distribution of the root biomass in different soil layers.

At the community level, all scenarios where one functional group was sown before the other two had similar shoot and root productivity. At the species level, two forbs (*Achillea millefolium* and *Centaurea jacea*) benefited from arriving early, and one legume (*Trifolium pratense*) had a disadvantage when it was sown after the grasses. Priority effect treatments also affected the vertical distribution of roots. When grasses were sown first, plant communities rooted more shallowly (lower mean and maximum rooting depth) than when forbs or legumes were sown first. In addition, roots moved down the soil profile more slowly in grasses-first communities.

Our results highlight that plant functional group order of arrival in grassland communities can affect the vertical distribution of roots in the soil and this may have implications for species coexistence.

Keywords: deep learning, plant order of arrival, priority effects, rhizoboxes, root image analysis, rooting depth, species coexistence

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Introduction

The order and timing of past biotic (e.g. species immigration) and abiotic (e.g. disturbances) events during community assembly can affect the structure and functioning of plant communities (Ejrnaes et al. 2006, Martin and Wilsey 2012, Sarneel et al. 2016, Werner et al. 2016, Weidlich et al. 2017, Delory et al. 2019b). This historical contingency may be caused by priority effects, in which the order of arrival of species determines how species interact and affect each other in communities (Fukami et al. 2010, Kardol et al. 2013, Vannette and Fukami 2014, Werner and Kiers 2015, Temperton et al. 2016). Because of differences in species traits such as seed germination, phenology and growth rates, priority effects are the norm rather than the exception in plant communities. If priority effects are strong enough to influence species interactions, they can lead to alternative stable states (Sutherland 1974), alternative transient states (Fukami and Nakajima 2011) and compositional cycles (Steiner and Leibold 2004), which are all possible long-term consequences of priority effects (Chase 2003, Fukami 2015).

In grasslands, priority effects have been studied mainly for their potential as a tool for ecological restoration to manipulate interactions between native and non-native species or as a way to foster ecosystem multifunctionality (Young et al. 2017, Hess et al. 2019, Weidlich et al. 2021). So far, most of the controlled and field experiments that have manipulated the sequence of arrival of species or plant functional groups (PFGs) have found strong aboveground priority effects on species diversity and aboveground productivity (Martin and Wilsey 2012, von Gillhaussen et al. 2014, Weidlich et al. 2017), with implications for the resistance of plant communities to invasion (Delory et al. 2019a, Hess et al. 2020, Yannelli et al. 2020). However, much less is known about plant order of arrival effects on the productivity and distribution of roots in different soil layers, at both the community and species level. Gaining such new knowledge is important because it would improve our understanding of plant community assembly and species coexistence, but also because it could be used in restoration to create deeper-rooted communities that can cope better with extreme weather events such as drought.

The overall productivity of roots and their distribution in the soil are the result of two main factors: 1) the anatomical, morphological and architectural characteristics of the species populating the community (e.g. plant size and vigour, root growth form, root traits, etc.), and 2) the phenotypic plasticity of the roots of individual species in response to environmental factors (e.g. plant neighbour identity and density, species richness, resource availability, soil texture, etc.) (Poorter et al. 2015, Herben et al. 2018, Bakker et al. 2019, Case et al. 2020, Chen et al. 2020, Lepik et al. 2021, Weigelt et al. 2021). To date, much of what we know about the biotic and abiotic factors affecting the production and distribution of root biomass in grassland soils comes from experiments that did not manipulate the timing and/or order of plant species arrival. For instance, the effect of plant species and functional group richness on root dynamics and distribution at the community and species level has been a very active area of research in ecology (de Kroon et al. 2012). Biodiversity–ecosystem functioning (BEF) experiments that manipulated plant diversity without manipulating plant arrival order often found that root biomass production increases with plant species richness (Mommer et al. 2010, Mueller et al. 2013, Ravenek et al. 2014, Jesch et al. 2018, Oram et al. 2018, Zeng et al. 2021). However, the effect of plant diversity on the vertical distribution of roots has not been as clear (Mommer et al. 2010, de Kroon et al. 2012, Luo et al. 2021). Recently, two meta-analyses showed that increasing plant species richness does not seem to affect the mean rooting depth of plant communities (Barry et al. 2020, Peng and Chen 2021). With the exception of one study that reported that high diversity plots in a grassland field experiment had a higher proportion of root biomass allocated to deeper soil layers (Mueller et al. 2013), there is little evidence supporting an increase in vertical root niche differentiation with increased plant diversity in either grasslands (Mommer et al. 2010, Ravenek et al. 2014, Oram et al. 2018) or forests (Valverde-Barrantes et al. 2015, Luo et al. 2021, Zeng et al. 2021). However, most BEF experiments did not manipulate the sequence of arrival of species, which may have important consequences for root dynamics and distribution in plant communities.

If species were to arrive sequentially, and not at the same time, one can expect the productivity and distribution of roots to be affected by plant order of arrival. For instance, if species A arrives before species B at a site, species A will start taking up resources earlier and will reduce the availability of essential soil resources for species B (niche preemption) (Kardol et al. 2013). In addition, species A may create soil legacies (niche modification) that will affect the establishment of species B (Kardol et al. 2007, Grman and Suding 2010, Delory et al. 2021). Both niche preemption and niche modification mechanisms could trigger plastic root responses in some later arriving species, which could affect how they develop and position their roots in the soil (Chen et al. 2020).

So far, both controlled and field experiments have revealed that manipulating the order of arrival of PFGs can affect root productivity in the topsoil of grasslands, with lower standing root biomass and root length density in the first 20 cm of soil when leguminous species were sown first (Körner et al. 2008, Weidlich et al. 2018a). If legumes are given a head start, their capacity to fix atmospheric N_2 could promote N facilitation mechanisms (i.e. N sparing and transfer) that could lead later-arriving species to invest more in shoots and less in roots (lower root:shoot ratio), which could explain the lower root productivity in the topsoil when legumes are sown first. However, this pattern could also be explained by a change in the vertical distribution of roots and a greater allocation of root biomass in deeper soil layers.

Although some studies found no difference in rooting patterns between grasses and forbs (Mommer et al. 2010, Ravenek et al. 2014, Oram et al. 2018), others reported that grasses tend to root more superficially (Bakker et al. 2019, 2021, Chen et al. 2020). If deep-rooting species (e.g. forbs and

legumes) arrive first at a site, they can take up resources from deeper soil layers and increase the amount of soil resources available in the topsoil for shallow-rooted species, which may then invest less in soil exploration (lower root:shoot ratio), leading to lower root productivity in the topsoil. In contrast, if early-arriving species have shallow roots (e.g. grasses), they will mainly pre-empt resources and modify the soil environment near the surface. Reduced availability of resources in the topsoil and/or soil legacy effects could then have a negative effect on the growth rates of later arriving species, resulting in a slower progression of roots in the soil profile and leading to a shallower root distribution. So far, it is not clear whether the differences in root productivity that have been observed by Körner et al. (2008) and Weidlich et al. (2018a) when the order of arrival of plants is manipulated are due to changes in total root productivity, or changes in the vertical distribution of roots, or both.

In this paper, we present the results of a controlled grassland experiment designed to test how PFG order of arrival (forbs, grasses and legumes) in mesic grasslands affects root productivity and the vertical distribution of roots at the early stages of plant community assembly. Our experiment tested the following hypotheses:

1) The above- and belowground productivity of plant communities varies depending on whether forbs, grasses or legumes were sown first. In particular, we expect to find higher total shoot productivity and lower root productivity when legumes were sown first.
2) The vertical distribution of roots depends on PFG order of arrival during assembly, with communities in which forbs or legumes were sown first moving down the soil profile more quickly and eventually rooting deeper than communities in which grasses were sown first.

Material and methods

Experimental design

We conducted a rhizobox experiment at a greenhouse located in Lüneburg, Germany (53°14′23.8″N, 10°24′45.5″E) in August–September 2017. We manipulated assembly history by altering the order of arrival of three plant functional groups (PFGs): non N_2-fixing forbs (forbs), N_2-fixing forbs (legumes) and grasses. Each plant community consisted of nine species that commonly co-occur in mesic grasslands: three forbs (*Achillea millefolium*, *Leucanthemum vulgare*, *Centaurea jacea*), three legumes (*Lotus corniculatus*, *Medicago sativa*, *Trifolium pratense*) and three grasses (*Dactylis glomerata*, *Festuca rubra*, *Holcus lanatus*).

In this experiment, we manipulated assembly history using five different PFG order of arrival scenarios: synchronous 1 (Sync1; all PFGs were sown simultaneously at the first sowing event), synchronous 2 (Sync2; all PFGs were sown simultaneously at the second sowing event), forbs-first (F-first; forbs sown before grasses and legumes), grasses-first (G-first; grasses sown before forbs and legumes) and legumes-first (L-first; legumes sown before forbs and grasses). Each PFG order of arrival scenario was replicated 7 times. Note that we set up two synchronous treatments so that the performance of plants that were sown at the first or second sowing event could be compared directly to plants that had grown for the same length of time. The time interval between the first and second sowing events was 10 days.

Experimental setup

One week before starting the experiment, 35 rhizoboxes (size of the soil compartment: 58 × 26.6 × 2 cm, height × width × thickness) were filled with a 5 mm-sieved mixture of sand (30%, v/v) and potting soil (70%, v/v; blend of weakly decomposed white sphagnum peat and clay supplemented with 1 kg m^{-3} of substrate fertilisers (14% N, 16% P_2O_5, 18% K_2O) and 2 kg m^{-3} of a slow-release fertiliser (NPK: 20-10-15)). Each rhizobox was equipped with a transparent front window that allowed us to non-destructively monitor root development over time. Rhizoboxes were watered with 100 ml of tap water and randomly assigned to an experimental treatment. Four days later, all rhizoboxes again received 50 ml of tap water. Seeds of the species belonging to the early-arriving PFG were sown one week after filling the rhizoboxes with soil. Ten days after this first sowing event, seeds of the species belonging to the late-arriving PFGs were added to the rhizoboxes. In synchronous treatments, all seeds were added on the same day (either at the first or second sowing event). The position of the functional groups inside the rhizoboxes was fixed (Fig. 1), but the position of each species within a functional group was assigned randomly. In each rhizobox, all plant individuals were equidistantly spaced (2.7 cm). A few days after each sowing event, the seeds that did not germinate were replaced by seedlings of the same species that had been allowed to germinate in petri dishes filled with the same soil as the one used in the rhizoboxes. The petri dishes were stored vertically in a plastic tray next to the experiment.

In our experiment, legumes, forbs and grasses started to germinate on average 2.3, 3.3 and 4.3 days after planting, respectively. This means that the seedlings of the last sown PFG started growing between 8 and 12 days after the seedlings of the first sown PFG. Each rhizobox was watered regularly with 20–50 ml of tap water. Over the entire duration of the experiment, each rhizobox received a total of 770 ml of tap water. All rhizoboxes were placed in plastic containers (5 rhizoboxes per container) and were inclined at a 45° angle in order to allow the roots to grow along the transparent front window. In each container, the front window of the first rhizobox was covered with a black plastic plate to prevent root exposure to light, and the back of the last rhizobox was covered with a white polystyrene plate to avoid overheating. The position of the rhizoboxes inside the greenhouse was regularly randomised during the experiment. Plants were harvested 44 days after the first sowing event. Both the length of the sowing interval (10 days) and the duration of the experiment were mainly limited by the size of our rhizoboxes and the fact

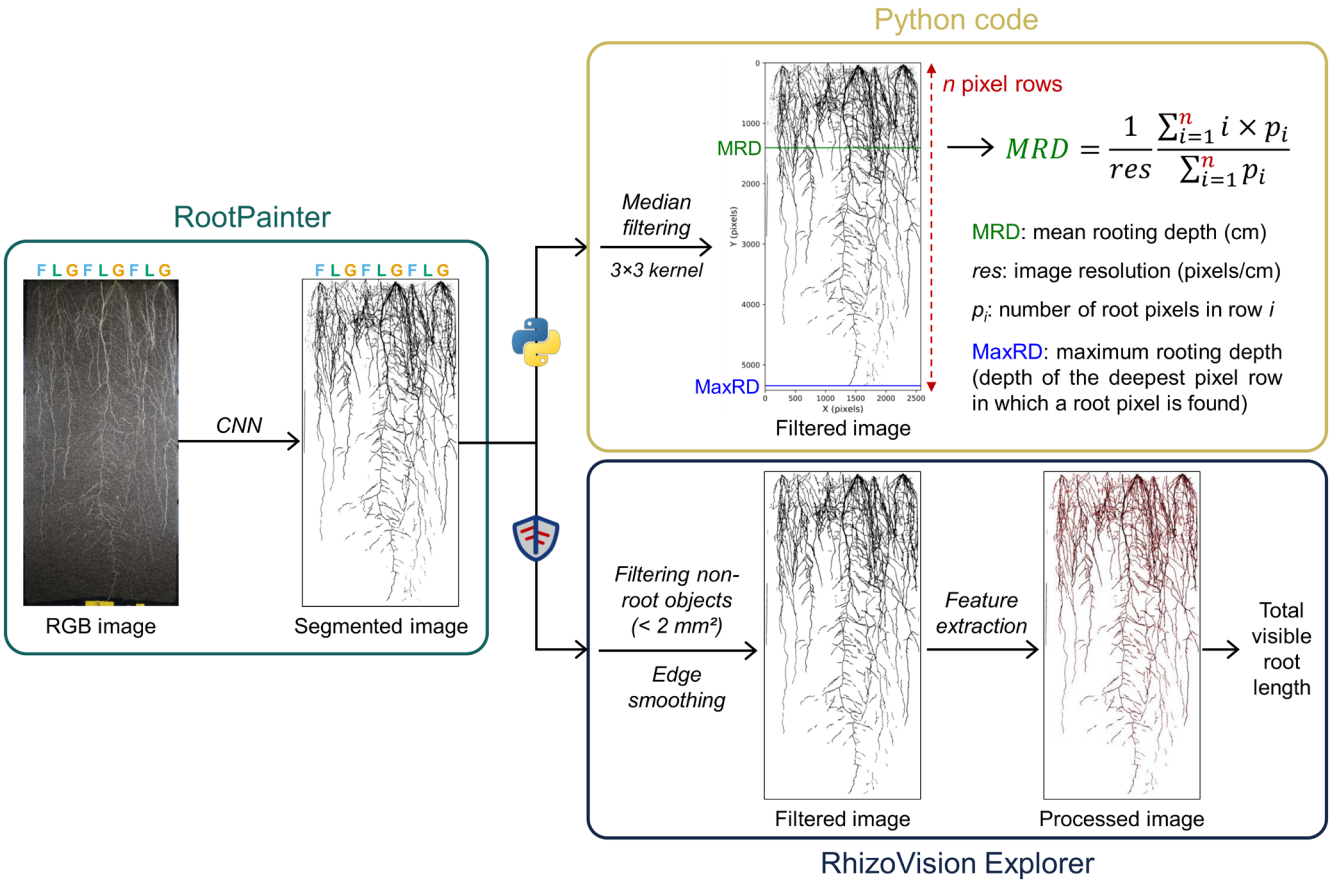


Figure 1. Description of the image analysis pipeline used for non-destructively estimating the total visible root length and the mean (MRD) and maximum (MaxRD) rooting depth of plant communities growing in rhizoboxes. Our approach included four main steps: 1) training a convolutional neural network (CNN) using RootPainter to detect plant roots in our images, 2) segmenting all images taken during the experiment with the best performing model, 3) estimating the mean and maximum rooting depth of each plant community at each time point using a Python procedure run in a Jupyter Notebook and 4) estimating the total visible root length in each image using RhizoVision Explorer. F, forb species; G, grass species; L, legume species.

that some of our species reached the bottom of the rhizoboxes after nearly 30 days.

Root image acquisition

Root images were acquired three times a week (Monday, Wednesday, Friday) using a digital camera equipped with a 28 mm lens and connected to a computer (Delory et al. 2018). In addition to the camera and its associated holder, our image acquisition system consists of a metallic frame holding the rhizobox vertically and two LED tubes (4300 K, 60 cm length) positioned laterally (raking lighting) to provide uniform lighting conditions over the entire height of a rhizobox. The camera, the camera holder, the LED tubes and the frame holding the rhizobox were installed inside a closed box whose internal walls were entirely covered with dark fabric. The camera faced each rhizobox directly and the camera lens was located 54 cm from the surface of the transparent front window. Images (3840 × 5760 pixels, width × height; resolution: 94.8 pixels cm^{-1} or ~240 dpi) were acquired using our camera's remote live view and shooting option before being saved on a computer using both compressed (jpeg) and uncompressed (raw CR2) file formats.

Harvest and measurements

Forty-four days after the start of the experiment (first sowing event), plant shoots were harvested and stored separately for each species. By this time, the roots of some plant species in the Sync1, L-first and F-first treatments had already reached the bottom of the rhizoboxes. Shoot samples were dried at 60°C until constant mass was reached, and weighed.

At harvest, rhizoboxes were opened by carefully removing the transparent front window. Using a sharp knife, the soil profile was divided into six 10-cm layers (0–10, 10–20, 20–30, 30–40, 40–50, > 50 cm). For each soil layer, roots were extracted from the soil under running water and stored at −20°C until further processing. Fine root washing was performed in the lab by carefully removing soil particles adhering to the roots (Delory et al. 2018). Clean root samples were then dried at 60°C for at least 48 h, and weighed. For each rhizobox, total root productivity was calculated

by summing the root dry weight values measured in all soil layers.

Image analysis

The image analysis pipeline used to analyse the root images taken in our experiment is shown in Fig. 1. First, root images were cropped in ImageJ using a custom macro to select only the zone of soil containing roots (Schindelin et al. 2012). These cropped images were used to create a training dataset of 600 smaller images (height: 612–990 pixels; width: 643–872 pixels). This training dataset was created with RootPainter in two steps: 1) randomly selecting 300 images from the 665 images taken during the experiment (35 rhizoboxes × 19 time points), and 2) randomly selecting two subregions of each image selected in step 1 (Smith et al. 2020). Then, RootPainter was used to annotate a number of training images and train a convolutional neural network (CNN) to detect roots in our images (Smith et al. 2020). Once model predictions successfully identified most of the roots present in our training images, the training was stopped and the best performing model was used to segment the cropped version of the 665 images taken during the experiment. The images segmented by RootPainter were then used to estimate the total visible root length and the mean (MRD) and maximum (MaxRD) rooting depth of each plant community at each time point. The total visible root length was estimated with RhizoVision Explorer ver. 2.0.3 using the batch-processing mode (thresholding level: 200; filter non-root objects larger than 2 mm^2; edge smoothing threshold: 1; root pruning threshold: 5) (Seethepalli et al. 2021). MRD was estimated as the depth value above which 50% of the roots were located (Mommer et al. 2010, Freschet et al. 2021), while MaxRD was estimated as the depth of the deepest pixel row in which a root pixel was found. MRD and MaxRD values were estimated using a custom Python procedure consisting of the following steps: 1) loading a segmented image and converting it to a binary format where a root pixel has a value of one and a background pixel has a value of zero, 2) applying a median filter (3 × 3 kernel) to remove noise and 3) calculating a value for MRD and MaxRD. For each image, MRD was calculated as the sum of the total number of root pixels in the *i*th pixel row multiplied by the depth of the *i*th pixel row divided by the total number of root pixels in that image (see equation shown in Fig. 1). At the last observation date (day 44), MRD values calculated by image analysis and MRD values calculated with root biomass data using the same approach were highly positively correlated (Supporting information).

In this paper, MRD and MaxRD were used as complementary measures to assess the vertical distribution of roots. While MRD is a relative value that informs about the depth above which half of the roots are located, it does not provide any information about the extent of root exploration with depth, which is provided by MaxRD. Here, we considered that a plant community A had a more shallow root distribution than a second community B if the MRD and MaxRD values measured in community A were located closer to the soil surface than the MRD and MaxRD values measured in community B.

Analysis of the vertical distribution of roots in the rhizoboxes

Two strategies were used to investigate the effect of PFG order of arrival on vertical root distribution: 1) modelling root biomass distribution in the soil (i.e. root dry weight as a function of depth), and 2) modelling the temporal evolution of the mean and maximum rooting depth of plant communities (i.e. rooting depth as a function of time).

Root biomass distribution in the soil was modelled using a zero-altered gamma model (ZAG model, also referred to as a hurdle model) following Zuur and Ieno (2016). This choice of statistical model was motivated by the fact that our dataset consisted of zero-inflated continuous data ($Y \geq 0$) since some layers did not contain any roots at harvest. The relationship between root biomass production and soil depth was investigated using a hurdle model combining a binomial generalised linear mixed-effect model (binomial GLMM) and a gamma generalised linear mixed-effect model (gamma GLMM). The binomial GLMM component was used to model the relationship between the presence/absence of roots ($Y = 1$ or 0) and soil depth, while the gamma GLMM part modelled the relationship between non-zero root biomass data ($Y > 0$) and soil depth. GLMMs were fitted using PFG order of arrival (5 levels), soil depth (continuous variable) and their interaction as fixed effects. To account for the fact that root biomass values measured at different depths in the same rhizobox were not independent, rhizobox ID was used as a random effect in the models (random intercept model). The binomial GLMM was fitted using a logit link function, while the gamma GLMM was fitted using a log link function. GLMM models were fitted using the MASS package in R (Venables and Ripley 2002).

The temporal evolution of MRD and MaxRD was modelled using linear mixed-effect models. The fixed component of the models contained PFG order of arrival (5 levels), the number of days after sowing each rhizobox (continuous variable) and their interaction. Considering that the mean and maximum rooting depth in each rhizobox was measured at multiple time points, the temporal evolution of MRD and MaxRD was modelled using a random slope and a random intercept for each rhizobox. The linear mixed-effect models were fitted using the lme4 R package (Bates et al. 2015).

Data analysis

As plant shoots were harvested separately for each species, shoot biomass data was analysed at both species and community levels. Root biomass data, however, was only analysed at the community level (roots from different species could not be separated from each other). When analysing and interpreting the data presented in this paper, we considered recent calls to stop using p-values in a dichotomous way and stop declarations of 'statistical significance' (Amrhein et al. 2019,

Wasserstein et al. 2019, Muff et al. 2021). To do so, we calculated effect sizes (difference between treatment means) and computed their 95% confidence intervals by bootstrap resampling (20 000 iterations). Following Amrhein et al. (2019), 95% confidence intervals will be referred to as compatibility intervals (CI) in the remainder of the paper. Since manipulating PFG order of arrival implies that seeds of different species were not sown at the same time in all rhizoboxes, meaningful comparisons had to be defined a priori so that the calculated effect sizes reflect plant order of arrival effects, and not differences in plant age. This means that the productivity of a species in a priority effect treatment (F-first, G-first or L-first) was compared either to the productivity achieved by the same species in the Sync1 treatment (if the target species was sown at the first sowing event), or Sync2 treatment (if the target species was sown at the second sowing event). For the total visible root length and shoot and root productivity data measured at the community level, effect sizes were calculated by comparing priority effect treatments to each other, but not to synchronous treatments. For each response variable, the strength of PFG order of arrival effects was assessed using non-standardised absolute effect sizes and their compatibility intervals (Amrhein et al. 2019, Rillig et al. 2019). Plots showing all computed effect sizes and compatibility intervals can be found in the HTML version of our R markdown file on Zenodo.

Data analysis was performed in R ver. 4.0.5 (www.r-project.org). Plots were created using the R packages ggplot2 (Wickham 2016), ggeffects (Lüdecke 2018), ggpubr (Kassambara 2020) and ggsignif (Ahlmann-Eltze and Patil 2021).

Results

Priority effects on shoot and root productivity

At the community level, we found that the total shoot productivity of F-first, G-first and L-first communities did not markedly differ from each other (Fig. 2a). Similarly, we did not find any difference in total root productivity between F-first, G-first and L-first communities (Fig. 2b). We found only weak evidence that the total length of roots visible through the transparent front window of the rhizoboxes differed between F-first, G-first and L-first communities (Fig. 2c–d). At harvest, the total visible root length was on average 2.1 m (+20%) to 2.9 m (+30%) longer in F-first communities than in G-first and L-first communities, respectively (Fig. 2d). As expected, the total visible root length and total aboveground and belowground productivity were highest in the Sync1 treatment, but lowest in the Sync2 treatment (Fig. 2a–d).

At the species level, two forb species clearly benefited from arriving early. Both *Achillea millefolium* (+87%) and *Centaurea jacea* (+67%) were more productive aboveground in the F-first treatment than in the Sync1 treatment (Fig. 3). In addition, one legume species, *Trifolium pratense*, suffered from arriving after the grasses. In the G-first scenario, *T. pratense* had a lower shoot biomass productivity than in the Sync2 (−34%) and F-first (−24%) treatments.

In order to understand why *A. millefolium* and *C. jacea* performed better aboveground when forbs were sown first, we investigated whether the biomass of these two species was negatively correlated with the biomass of other species present in Sync1 rhizoboxes. We found that the biomass of *A. millefolium* was weakly negatively correlated to the biomass of *Medicago sativa* ($r=-0.71$, $p=0.075$), which was the most productive species in synchronous treatments. We did not find evidence for a negative relationship between the biomass of *C. jacea* and *M. sativa* ($r=0.08$, $p=0.858$). Interestingly, *A. millefolium* was *M. sativa*'s direct neighbour in 3 replicates out of 7 (the two lowest biomass values for *A. millefolium* were measured in these rhizoboxes), while *C. jacea* was *M. sativa*'s direct neighbour in only one rhizobox. The legume *Lotus corniculatus* was *C. jacea*'s direct neighbour in 6 replicates out of 7, but we only found a weak negative correlation between the biomass of these two species in Sync1 rhizoboxes ($r=-0.49$, $p=0.268$). The biomass of each grass species was poorly correlated to the biomass of *A. millefolium* and *C. jacea*.

Root biomass distribution in the soil

Despite the fact that we did not observe any difference in total root productivity between the F-first, G-first and L-first scenarios, we found that the vertical distribution of root biomass in the soil was strongly affected by PFG order of arrival (Fig. 4, Supporting information). When grasses were sown first (G-first), a larger proportion of the root biomass was found at the top of the soil, and the observed decrease in root productivity with depth was stronger than when forbs or legumes were sown first (Fig. 4, Supporting information). In fact, we did not find any roots located deeper than 50 cm when grasses were sown first (Supporting information). This was not the case when forbs or legumes were sown first since roots located deeper than 50 cm were mostly present (all but one rhizobox) (Supporting information).

Temporal evolution of the mean and maximum rooting depth of plant communities

Our results showed that the temporal evolution of the mean (MRD) and maximum (MaxRD) rooting depth of plant communities was dependent on PFG order of arrival (Fig. 5). Roots moved down the soil profile more slowly for G-first communities. This is shown by a reduction of the slope of the G-first line in Fig. 5b. On average, MRD increased by 2.7 mm day^{-1} in synchronous (Sync1 and Sync2), F-first and L-first communities, whereas it increased by only 2.1 mm day^{-1} in G-first communities, which represents a 23% decrease in progression rate (Fig. 5a–b). We observed an even stronger effect of PFG order of arrival on the maximum rooting depth (Fig. 5c–d). On average, MaxRD increased by 1.8 cm day^{-1} in Sync1 and L-first communities, whereas it increased by

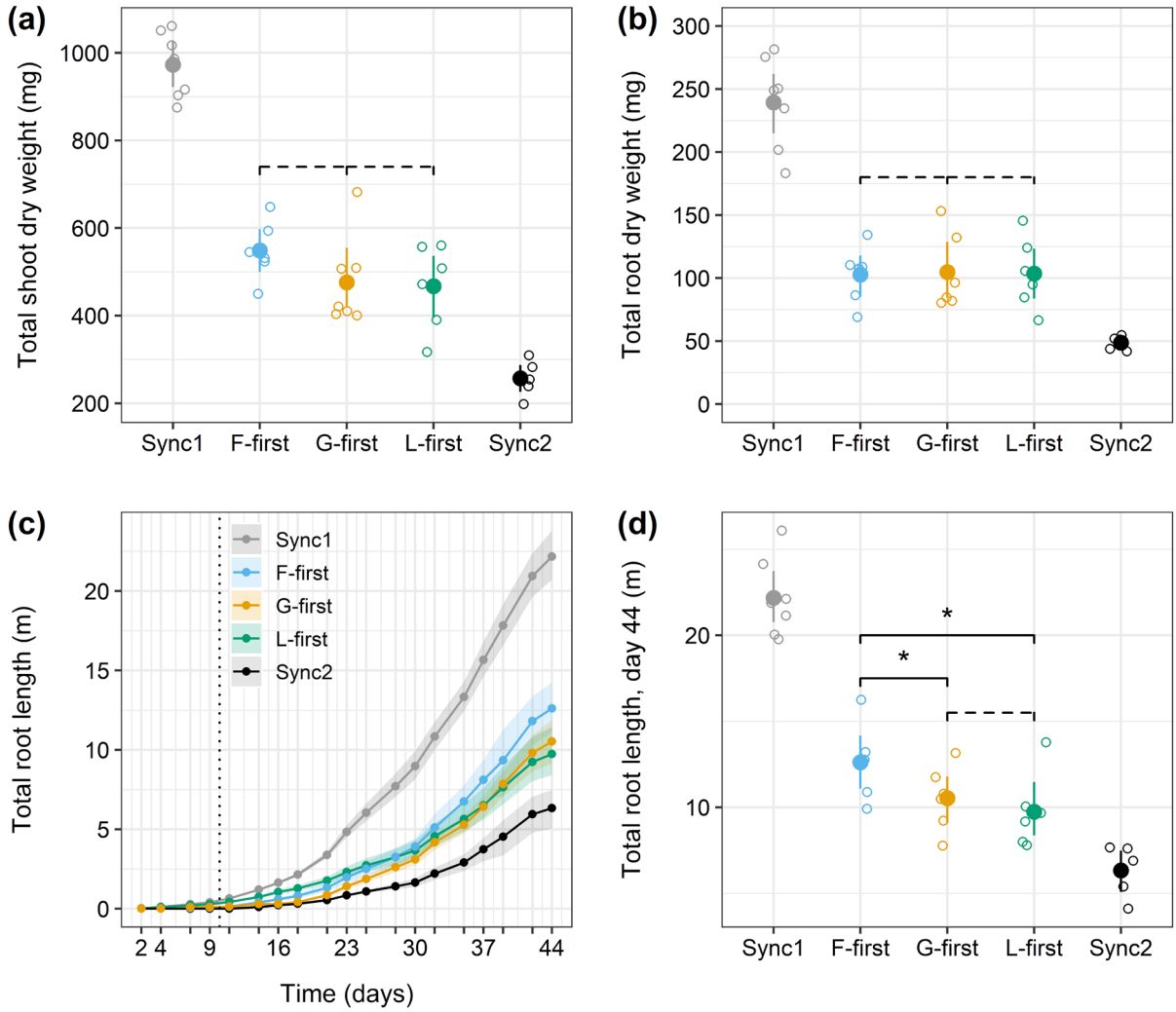


Figure 2. Effect of PFG order of arrival on the total shoot productivity (a), total root productivity (b) and total visible root length (c–d) of plant communities. For each treatment, mean values (solid dots) and compatibility intervals (solid bars in panels a, b and d; shaded areas in panel c) computed using non-parametric bootstrap are shown (n = 5–7). Individual observations are displayed as open dots. An asterisk (*) indicates that our data were weakly compatible with an effect size of zero, which supports the existence of a small difference in productivity or total visible root length between groups. Comparisons for which our data were compatible with an effect size of zero (i.e. no difference in productivity or total visible root length) are represented by dashed black lines. Effect sizes and compatibility intervals can be found in the HTML version of our R markdown file on Zenodo (Alonso-Crespo et al. 2021). Sync1, all PFGs sown at the same time at the first sowing event; F-first, forbs sown 10 days before grasses and legumes; G-first, grasses sown 10 days before forbs and legumes; L-first, legumes sown 10 days before forbs and grasses; Sync2, all PFGs sown at the same time at the second sowing event.

only 1.5 cm day^{-1} in F-first communities (−15%), 1.2 cm day^{-1} in Sync2 communities (−33%) and 0.95 cm day^{-1} in G-first communities (−48%) (see slope changes in Fig. 5d).

At the last observation date (day 44), the mean rooting depth measured in Sync1, F-first and L-first communities was located between 3.1 and 4.6 cm deeper than G-first communities (Fig. 5a–b). At day 28 (i.e. before the roots of some species reached the bottom of the rhizoboxes), the maximum rooting depth measured in G-first and Sync2 communities was located between 16.0 and 25.6 cm above the maximum rooting depth measured in Sync1, F-first and L-first communities.

Discussion

We found no difference in total shoot and root biomass production between communities in which grasses, forbs or legumes were sown first. Nevertheless, our results showed that sowing grasses before forbs and legumes led to more shallow-rooted communities, supporting the fact that assembly history can modulate the vertical distribution of roots in grassland ecosystems. This result was apparent for two reasons. Firstly, we found that grasses-first communities had a greater proportion of their root biomass near the soil surface. Second, we found that roots moved more slowly down

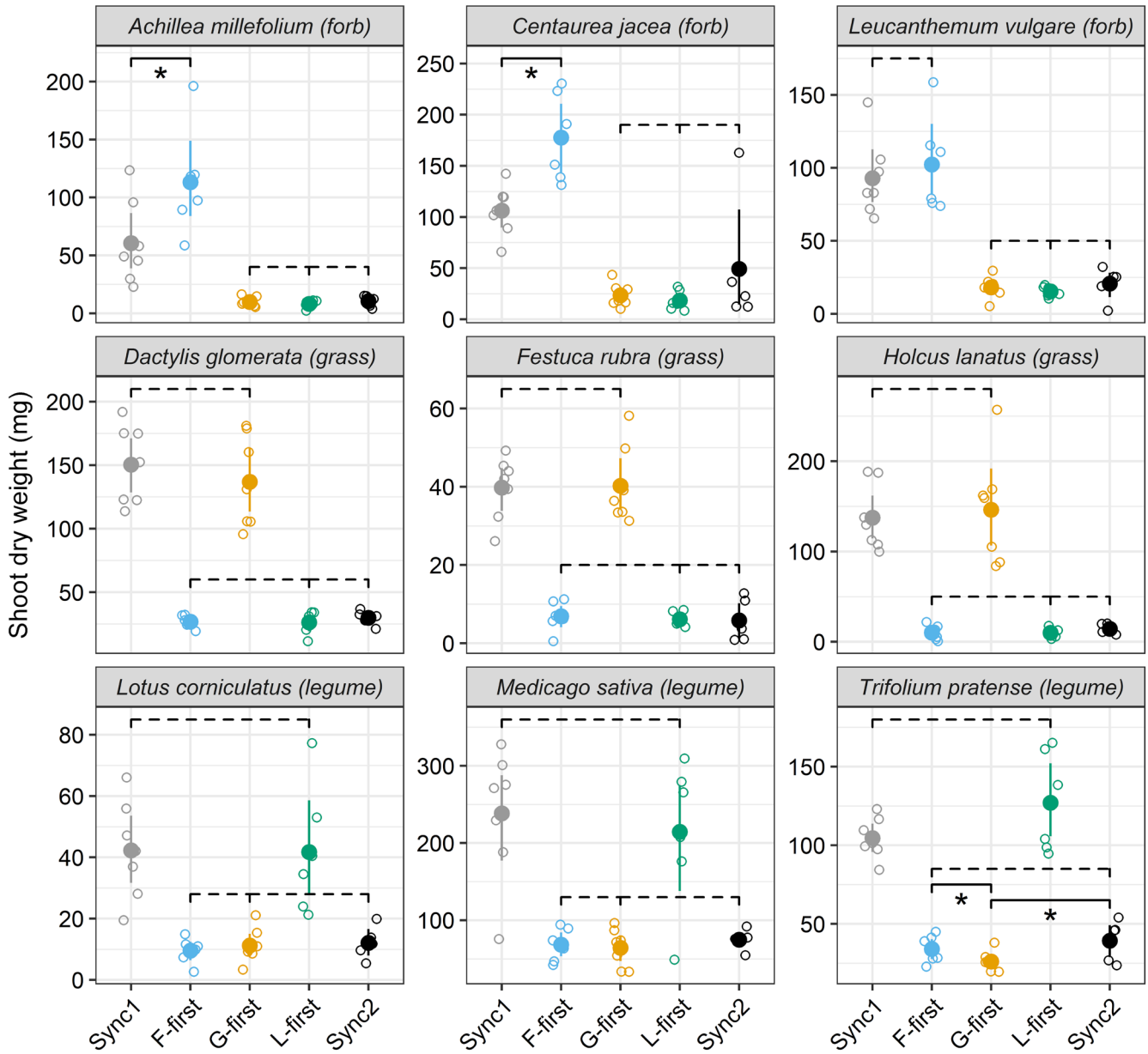


Figure 3. Effect of PFG order of arrival on the shoot productivity of individual species. For each treatment, mean values (solid dots) and compatibility intervals (solid bars) computed using non-parametric bootstrap are shown (n = 5–7). Individual species observations are displayed as open dots. In all panels, meaningful comparisons are shown with solid and dashed black lines. An asterisk (*) indicates that our data were not compatible with an effect size of zero, which supports the existence of a difference in total shoot productivity between groups. Comparisons for which our data were compatible with an effect size of zero (i.e. no difference in total shoot productivity) are represented by dashed black lines. Effect sizes and compatibility intervals can be found in the HTML version of our R markdown file on Zenodo (Alonso-Crespo et al. 2021). Sync1, all PFGs sown at the same time at the first sowing event; F-first, forbs sown 10 days before grasses and legumes; G-first, grasses sown 10 days before forbs and legumes; L-first, legumes sown 10 days before forbs and grasses; Sync2, all PFGs sown at the same time at the second sowing event.

the soil profile in communities where grasses were sown first, leading these communities to have lower mean and maximum rooting depths at harvest.

At the community level, shoot and root productivity were similar if forbs, grasses or legumes were sown first. This result contradicts our first hypothesis that L-first communities have higher shoot productivity and lower root productivity. These results can appear surprising because they contradict findings from other controlled experiments that manipulated PFG order of arrival and found that grassland plant communities in which legumes were sown a few weeks before grasses and forbs were amongst the most productive aboveground (Körner et al. 2008, von Gillhaussen et al. 2014, Weidlich et al. 2017), but the least productive belowground

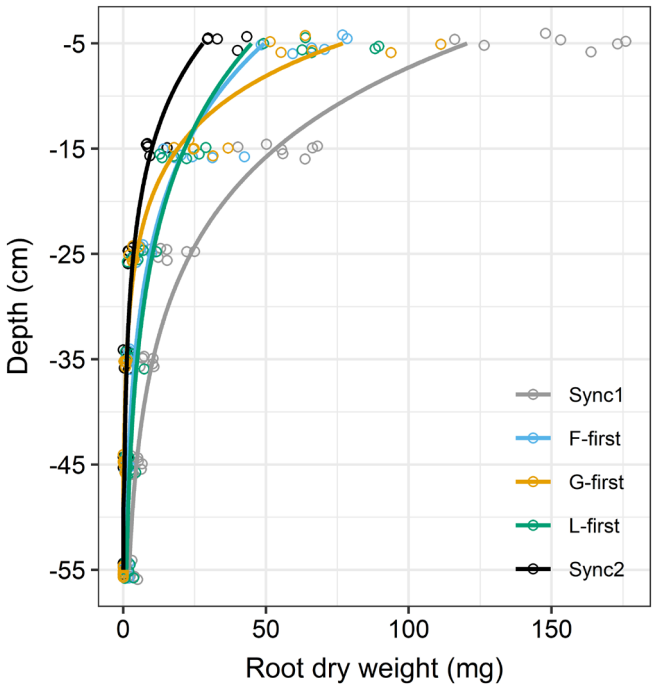


Figure 4. Vertical root biomass distribution in the soil is affected by PFG order of arrival. Lines represent ZAG model fits for each PFG order of arrival scenario (i.e. predictions obtained after merging the gamma and binomial components of the ZAG model). Predictions of the gamma and binomial components of the ZAG (or hurdle) model are shown in the Supporting information. Root biomass production values are shown for each soil layer (n = 5–7). Individual observations are displayed as open dots. Sync1, all PFGs sown at the same time at the first sowing event; F-first, forbs sown 10 days before grasses and legumes; G-first, grasses sown 10 days before forbs and legumes; L-first, legumes sown 10 days before forbs and grasses; Sync2, all PFGs sown at the same time at the second sowing event.

(Körner et al. 2008, Weidlich et al. 2018a). We suggest that differences in experimental conditions (species composition, sowing interval, plants grown in rhizoboxes, etc.) as well as the fact that our experiment focused on the early stages of plant community assembly are likely to be among the main factors explaining why our study yielded unusual results regarding how PFG order of arrival affected the productivity of plant communities. As our experiment focused on early shoot and root development, we cannot exclude the fact that differences in shoot and/or root productivity might have been more apparent if our experiment had lasted longer. In addition, the use of a larger species pool and the inclusion of species with stronger competitive abilities could also lead to stronger arrival order effects.

Our results suggested that weaker competition for space and resources with legumes (mainly *M. sativa*), especially aboveground, may be an important mechanism explaining the better performance of forbs (mainly *A. millefolium*) when they were sown first. Another possible explanation for the fact that *C. jacea* and *A. millefolium* benefited from an early arrival (while *L. vulgare* did not) could be related to their different rooting strategies and competitive ability. In a pairwise competition experiment, Ravenek et al. (2016) found that *L. vulgare* was a superior competitor than *C. jacea*, which could help explain why *L. vulgare* had a similar productivity when it arrived earlier or at the same time as grasses and legumes. Furthermore, in comparison with *C. jacea* and *A. millefolium*, *L. vulgare* seems to have another rooting strategy that aims to explore the topsoil more efficiently by producing longer and finer roots (higher SRL) and invest less biomass in deeper soil layers (lower deep root fraction) (Bakker et al. 2019). In a common garden experiment, Bakker et al. (2021) also found that deep-rooting species, such as *C. jacea*, benefited from growing next to heterospecific neighbours with shallow roots, while the performance of shallow-rooted species, such as *L. vulgare*, was not related to the deep root fraction of their neighbours. Considering that *C. jacea* and *A. millefolium* grew better when they were sown first, our results suggest that root traits such as deep root fraction may be important in mediating the susceptibility of plant species to plant order of arrival, with deep-rooting species benefiting more from arriving early than shallow-rooting species. In support of this hypothesis, previous studies have shown that the competitive superiority of grassland species was positively correlated with traits related to plant size and soil exploration, such as rooting depth, relative growth rate and root length density (Kraft et al. 2015, Ravenek et al. 2016). Because of their capacity to efficiently explore the topsoil and quickly access and take up essential resources, shallow-rooting species with a high SRL can have a competitive advantage over species with a lower SRL near the soil surface. If this is true, we would expect that deep-rooting species with a low SRL arriving a few days earlier than other species in the community would benefit from this situation, as they will suffer less from interspecific competition with their neighbours, access more resources, have higher growth rates and, ultimately, higher productivity than if they arrive at the same time as the other species. Whether or not root traits play a role in determining the susceptibility of plant species to plant order of arrival deserves further investigation.

Our results showed that manipulating PFG order of arrival had a strong effect on the vertical distribution of roots in the soil. We found that communities in which forbs or legumes were sown first rooted deeper (greater MRD and MaxRD) than communities in which grasses arrived a few days before the other PFGs, thus confirming our second hypothesis. If the shallower vertical root distribution in grasses-first communities led to an increase in interspecific competition for resources in the topsoil, it may be a possible explanation for the lower shoot productivity of *T. pratense* when it was sown after the grasses. Although strong differences in vertical root distribution exist between individual plant species (Herben et al. 2018, Lepik et al. 2021), evidence for differences in root distribution and competitive ability between plant functional groups in grassland ecosystems has been mixed, with some studies reporting no difference in rooting depth and competitive ability between functional groups (Mommer et al. 2010, Ravenek et al. 2014, 2016, Oram et al. 2018), while others

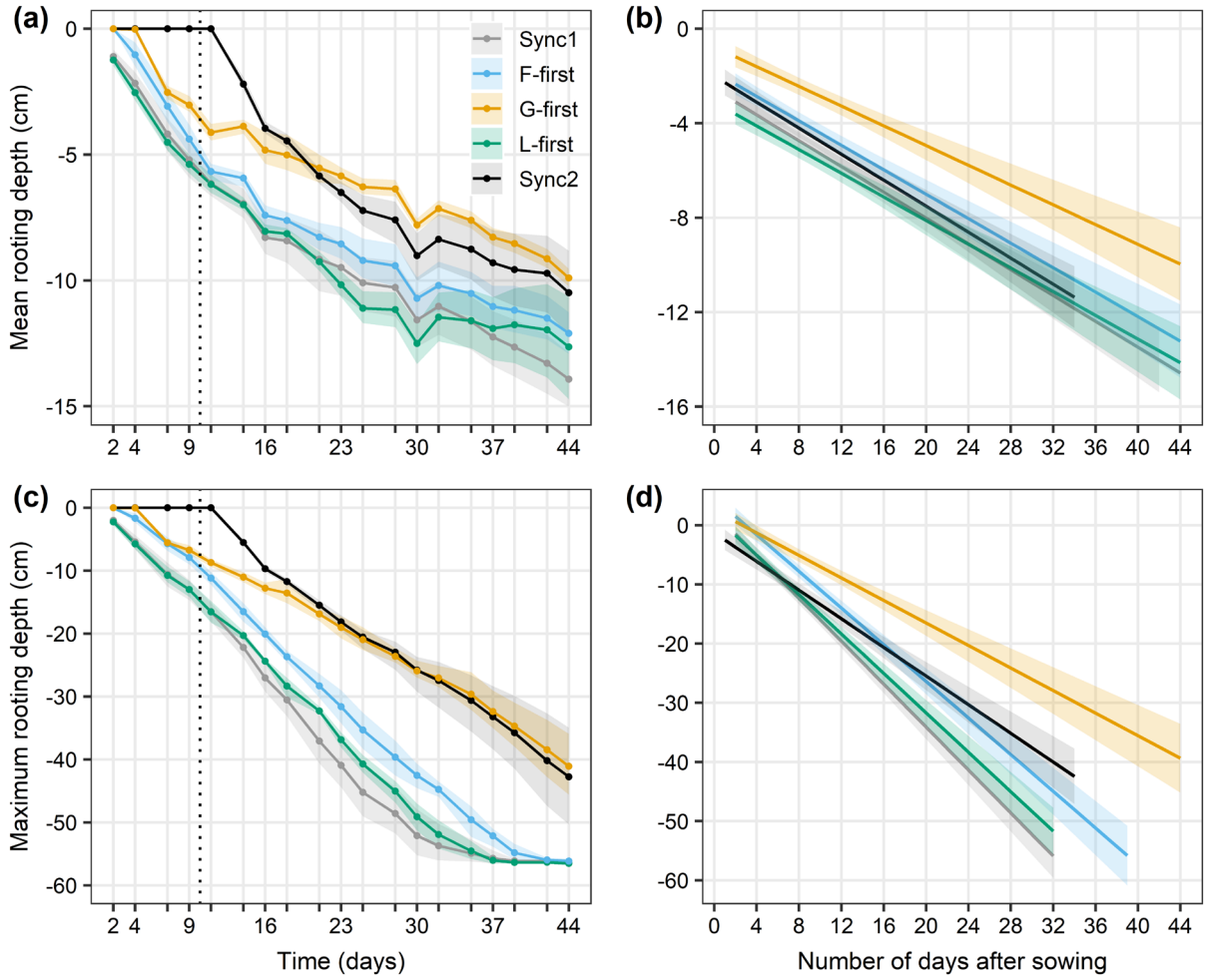


Figure 5. The temporal evolution of rooting depth is affected by PFG order of arrival. (a) Temporal evolution of the mean rooting depth (MRD) of plant communities for each PFG order of arrival scenario; (b) linear mixed-effect model fits describing the temporal evolution of MRD for each plant community type; (c) temporal evolution of the maximum rooting depth (MaxRD) of plant communities for each PFG order of arrival scenario; (d) linear mixed-effect model fits describing the temporal evolution of MaxRD for each plant community type. In (a) and (c), average MRD and MaxRD values are represented at each time point as solid dots (n = 5–7), while compatibility intervals computed using non-parametric bootstrap are shown as shaded areas. The dotted vertical line in (a) and (c) marks the day of the second sowing event (10 days after the start of the experiment). Sync1, all PFGs sown at the same time at the first sowing event; F-first, forbs sown 10 days before grasses and legumes; G-first, grasses sown 10 days before forbs and legumes; L-first, legumes sown 10 days before forbs and grasses; Sync2, all PFGs sown at the same time at the second sowing event.

have indicated that grasses have a lower deep root fraction and root more superficially than forbs (Bakker et al. 2019, 2021, Chen et al. 2020). Since our plant communities had exactly the same species and functional group composition and only differed by the order of arrival of forbs, grasses and legumes, differences in rooting depth between species or functional groups is unlikely to be the only explanation behind our results. Instead, plastic root responses to plant order of arrival are more likely to explain why grasses-first communities rooted more shallowly than the others did. Such plastic root responses affecting root allocation and foraging have been well documented in the past (Mahall and Callaway 1991, Semchenko et al. 2007, Mommer et al. 2012, Kumar et al. 2020, Lepik et al. 2021) but, to date, there has been little evidence that the order of arrival of plants can affect the root distribution of individual species (Weidlich et al. 2018b). These results highlight the need for more research on how plant order of arrival modulates root foraging and distribution at the functional group and species level, which is a particularly challenging endeavour.

Studying the extent to which the behaviour of the roots of species inhabiting plant communities changes as a function of the order of arrival of plants, as well as studying how these plastic root responses would be reflected at the community level, requires information on the distribution of roots at the species level, which unfortunately was not available in our study. Given the above, as well the inherent limitations of any rhizobox experiment, we see two main avenues for future research aimed at better understanding the roles played by priority effects in root dynamics and their consequences for

species coexistence: 1) non-destructively monitoring root development at different soil depths in the field using minirhizotrons (Rewald and Ephrath 2013, Freschet et al. 2021), and 2) quantifying species relative abundance in root samples taken from plant communities at different soil depths using state-of-the-art molecular techniques (Wagemaker et al. 2021). Going underground will provide us with a unique opportunity to improve our mechanistic understanding of priority effects in plant communities and their implications for species coexistence.

Conclusions

Using root biomass distribution data and deep learning-based image analysis, we provided evidence that assembly history, and more particularly plant order of arrival during assembly, can modulate the vertical distribution of roots in a controlled grassland experiment. When grasses were sown before forbs and legumes, plant communities had the shallowest root distribution. This result was explained by the fact that 1) a greater proportion of root biomass was present at the top of the soil, and 2) roots progressed more slowly through the soil in communities in which grasses were sown first. Further research is needed to better understand how priority effects alter root dynamics at the community and species level, and how this may affect species coexistence. Field experiments that use a larger sowing interval between plant functional groups would be ideal for such an endeavour.

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Author contributions

Inés M. Alonso-Crespo: Formal analysis (equal); Investigation (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal). **Emanuela W. A. Weidlich**: Conceptualization (equal); Investigation (equal); Writing – review and editing (equal). **Vicky M. Temperton**: Conceptualization (equal); Supervision (equal); Writing – review and editing (equal). **Benjamin M. Delory**: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal); Supervision (lead); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Zenodo Digital Repository: https://doi.org/10.5281/zenodo.5713397 (Alonso-Crespo et al. 2021).

Supporting information

The supporting information associated with this article is available from the online version.

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