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Exploring Priority and Year Effects on Plant Diversity, Productivity and Vertical Root Distribution: First Insights From a Grassland Field Experiment

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ABSTRACT

Question: The order of arrival of plant species during community assembly can affect how species interact with each other. These so-called priority effects can have strong implications for the structure and functioning of plant communities. However, the extent to which the strength, direction, and persistence of priority effects are modulated by weather conditions during plant establishment ("year effects") is not well known.

Location: Niederhaverbeck, Bispingen, Germany.

Methods: We present the first results from a field experiment initiated in 2020 in Northern Germany to test how plant functional group (PFG) order of arrival and the year of initiation of an experiment interactively affect the structure and functioning of nutrient-poor dry acidic grasslands, both above and below ground. To do this, we established the same experiment, manipulating the order of arrival of forbs, grasses, and legumes on the same site, but in different years representing different weather conditions.

Results: We found that time since establishment was a stronger driver of plant community composition than PFG order of arrival and year of initiation. PFG order of arrival effects on plant diversity evolved over time and depended on the year of initiation of an experiment. Year of initiation, not PFG order of arrival, was the strongest driver of aboveground community productivity. Although we did not find an effect of PFG order of arrival on root productivity, it had a strong impact on the vertical distribution of roots. Communities where grasses were sown first rooted more shallowly than communities in which forbs or legumes were sown first.

Conclusions: In experimental dry acidic grassland communities, community composition and productivity are shaped by time since establishment and initial weather conditions, rather than PFG order of arrival (6-week sowing interval). Importantly, our results demonstrate that manipulating PFG order of arrival is possibly an effective restoration measure to alter vertical root distribution towards more deep-rooting communities when sowing forbs or legumes first. This in turn could benefit dry grasslands on sandy soils during periods of water deficit.

1 | Introduction

Community assembly has long been a focal point in ecological research, aimed at elucidating processes and mechanisms governing the success of species under specific circumstances within a plant community over time. Traditionally, community assembly theory has posited that vegetation composition at any given timepoint is the result of dispersal and environmental filtering (Kelt et al. 1995) as dominant factors that are modulated by biotic interactions (such as grazing or disease). There is mounting evidence, however, that challenges this common view, finding that historical factors can have a substantial influence on community assembly (Fukami 2015). For instance, Grman and Brudvig (2014) examined the relative importance of management, site, and history in 27 restored prairies and found that management and history played a more important role than site and landscape factors for restoration outcomes.

Priority effects occur when species that arrive and establish before others can significantly influence the establishment and success of species that arrive later (Drake 1991; Fukami 2015), thus also sometimes influencing ecosystem functioning (Fukami et al. 2010; Delory et al. 2019). Priority effects can lead to alternative vegetation states (Chase 2003; Fukami 2015) and thus may play a key role in conservation. Priority effects can occur naturally (and be stochastic) but a better knowledge of the mechanisms and strength of priority effects, as well as their context-dependence, could allow for deliberate management interventions that send communities on desired trajectories (Weidlich et al. 2021). Thus, an improved understanding of ecological history, including priority effects caused by differences in timing and order of arrival and establishment of species, will be a linchpin for improving the predictive power of community ecology.

Priority effect experiments that manipulate community assembly such that different plant functional groups (hereafter called PFG) arrive earlier than others have shown that the legacy of which PFG establish first at a site after disturbance can alter not only plant diversity but also productivity in grassland ecosystems (Körner et al. 2008; von Gillhaussen et al. 2014; Weidlich et al. 2017; Delory et al. 2019). Studies that have followed the PFG approach cluster species based on their traits or degree of phylogenetic relatedness and manipulate their order of arrival in grassland systems under controlled and field conditions (see Weidlich et al. (2021) for an overview). These studies found clear effects of PFG order of arrival on productivity, both aboveground (Körner et al. 2008; von Gillhaussen et al. 2014; Weidlich et al. 2017) and belowground (Körner et al. 2008; Weidlich et al. 2018), with communities where legumes were sown before the others often having higher aboveground productivity but lower root biomass in the topsoil, which could be due to nitrogen facilitation. In a previous short-term controlled experiment with rhizoboxes, we showed that manipulating PFG order of arrival can also affect the vertical distribution of roots in the soil, with communities where grasses were sown first rooting more shallowly than communities where either forbs or legumes were sown first (Alonso-Crespo et al. 2023). This pattern could be due to the shallow rooting strategy of grasses (Bakker et al. 2019, 2021; Chen et al. 2020), which could increase interspecific competition for resources in the topsoil and trigger plastic root responses affecting root allocation and foraging in later-arriving plants (Alonso-Crespo et al. 2023). It is not known whether these results are also valid under field conditions, which argues in favor of longer-term experiments to monitor root development and distribution, using minirhizotron tubes for example.

Despite the importance of time and persistence of priority effects for trajectories and alternative states of plant communities, little attention has so far been paid to either long-term or year effects (e.g., the environmental conditions in which the initial community assembly takes place) (Stuble et al. 2017b). Evidence for year effects has been hard to extract from studies due to correlational considerations and confounding factors (MacDougall et al. 2008; Groves et al. 2020), but Stuble et al. (2017a, 2017b) studied the effect of the year of initiation and site location on restoration outcomes and found that both strongly affected the strength of priority effects and community composition. Werner et al. (2020) went further by including two different sowing intervals (2 weeks and one year) within priority effect treatments across sites and initiated in different years. They found that the year effect was by far the largest driver of outcomes, followed by the length of the sowing interval between native and exotic species. This suggests that priority effects, although important, may be less important than local environmental conditions like climate and soil properties or management practices in affecting outcomes. Interannual variation in environmental conditions during plant establishment has also been shown to be an important driver of the taxonomic and functional composition and diversity of plant communities, with consequences for ecosystem functioning (Werner et al. 2020; Catano et al. 2023; Atkinson et al. 2023). Precipitation patterns are an important driver of year effects in plant community assembly (Stuble et al. 2017a), but interannual variation in environmental variables other than precipitation can play an equally important role in driving year effects during restoration (Groves and Brudvig 2019). To better predict restoration outcomes, it is necessary to delve deeper into the ecological mechanisms underlying year effects and their context-dependence (MacDougall et al. 2008; Groves and Brudvig 2019; Catano et al. 2023; Atkinson et al. 2023).

Although it has been shown that PFG order of arrival can affect community structure, above- and belowground productivity, and root distribution in the soil (Körner et al. 2008; von Gillhaussen et al. 2014; Weidlich et al. 2017, 2018; Alonso-Crespo et al. 2023), little is known about how persistent these priority effects are over time and how this is mediated by the environmental conditions during establishment. Additionally, belowground, it remains uncertain whether priority effects on root productivity in the topsoil are a consequence of changes in the total root productivity (i.e., in the entire soil profile) or changes in the vertical root distribution.

In this study, we present the first results of a long-term field experiment (POEM, PriOrity Effect Mechanisms) initiated in 2020 and designed to test how priority and year effects modulate the structure and functioning of dry acidic grassland plant communities over time, both aboveground and belowground. To do this, we set up independent sub-experiments at a site in Northern Germany, where we tested the same PFG order of arrival scenarios, but in different years. Here, we used species-specific shoot biomass data collected between 2020 and 2023, as well as root images taken at different depths using minirhizotrons between 2021 and 2023, to address the following hypotheses:

1. Manipulating PFG order of arrival affects species composition and plant diversity, with plant communities following different trajectories depending on the year of initiation of an experiment.
2. PFG order of arrival and year of initiation interactively affect the aboveground productivity of plant communities. Following previous work (Körner et al. 2008; Weidlich et al. 2017), we expect plant communities in which legumes were sown first to be the most productive, but not necessarily for each year of initiation.
3. Root productivity in the first 50 cm of soil depends on PFG order of arrival, with plant communities in which legumes were sown first being the least productive belowground (Körner et al. 2008; Weidlich et al. 2018).
4. PFG order of arrival affects the vertical distribution of roots at the community level. Following previous work (Alonso-Crespo et al. 2023), we expect communities in which forbs or legumes were sown first to root deeper than communities in which grasses were sown first.

2 | Materials and Methods

2.1 | Study Site

The POEM experiment is located on a former arable land owned by a local conservation organization (Verein Naturschutzpark, VNP) in a fenced area in Niederhaverbeck, Germany (latitude: 53.144272, longitude: 9.912668; altitude: 105 m a.s.l.; mean annual air temperature: 10.2°C; minimum air temperature: −14.9°C; maximum air temperature: 38.7°C; mean annual precipitation: 684 mm; based on data from a weather station installed at our experimental site, period: 2020–2023). The experiment was set up on a soil that is appropriate for establishing dry acidic grassland communities. Based on soil samples collected from the field at different time points, soil characteristics are as follows: sand fraction: 93%; clay and silt fraction: 4%; pH ($CaCl_2$) 4.9; organic matter content: 2.3%; total N: 0.07%; total C: 0.98%; C/N: 12.1. Our experimental site had been used during the last 200 years as a cultivated arable field. In the years preceding the experiment, the following species were grown in rotation on the agricultural field where the POEM experiment was set up: *Trifolium repens*, *Trifolium pratense*, *Trifolium incarnatum*, *Lolium perenne*, Festulolium (*Festuca* sp. × *Lolium* sp.) and *Secale cereale*. Existing vegetation was removed (hand weeding and harrowing) before experimental plots were set up.

2.2 | Species Pool and Classification Into Plant Functional Groups

Fourteen native plant species were used in this field experiment: four N_2-fixing legumes (*Lathyrus pratensis* L., *Lotus corniculatus* L., *Trifolium arvense* L., and *Trifolium campestre* Schreb.), four grasses (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Bromus hordeaceus* L., and *Festuca ovina* agg.), and six forbs (*Dianthus deltoides* L., *Jasione montana* L., *Pilosella officinarum* Vaill., *Pimpinella saxifraga* L., *Potentilla argentea* L., and *Silene vulgaris* (Moench) Garcke). These species were chosen based on typical plant functional group ratios found in dry acidic grasslands (i.e., more forbs than grasses and legumes), as well as their availability from a regional/local wild seed company. The seeds of all species were obtained from Rieger-Hofmann GmbH (Blaufelden, Germany). The taxonomy of the vascular plants follows Hand and Thieme (2024).

2.3 | Experimental Design

The POEM field experiment was set up using a full factorial design to test for the influence of (1) plant functional group (PFG) order of arrival and (2) the year of initiation of an experiment on the structure and functioning of dry grassland plant communities, both aboveground and belowground.

Year of initiation effects are tested by setting up the same experiment at the same site, but in different years. Our POEM field experiment therefore consists of multiple sub-experiments initiated in different years and testing the same PFG order of arrival scenarios with the same level of replication. Here, we used the results obtained for the first two sub-experiments set up in 2020 (referred to as POEM2020) and 2021 (referred to as POEM2021), respectively. A detailed overview of the experimental design for these first two sub-experiments is provided in Figure 1A. Two additional sub-experiments will be set up in the coming years. At our POEM site, sub-experiments are at least 8 m apart from each other. Given that (1) our experimental site has been used for the past 200 years as a cultivated field, (2) our experimental area is relatively small (< 0.27 ha), and (3) the pool of unsown species is much similar in the first two sub-experiments and mainly includes classical arable ruderal species, we do not expect the location of each sub-experiment on our experimental site to interact with the year of initiation of each sub-experiment.

In each POEM sub-experiment, we manipulated the order of arrival of forbs, grasses, and legumes. We tested five arrival scenarios: (1) simultaneous sowing of forbs, grasses, and legumes at the first sowing event (synchronous, S), (2) forbs sown 6 weeks before grasses and legumes (F), (3) grasses sown 6 weeks before forbs and legumes (G), (4) legumes sown 6 weeks before forbs and grasses (L), and (5) no sowing of additional species (free succession, initiated on bare soil; B) (Figure 1B). The synchronous scenario (i.e., no timing and order of arrival manipulation of sown species) is used as a reference to quantify priority effects in our experiment. The six-week sowing interval is identical to that used in a previous priority effect experiment in Germany (Weidlich et al. 2017), facilitating comparisons between two different grassland types. Each arrival scenario was replicated 5 times in each sub-experiment. Thus, each POEM sub-experiment consists of 25 mixture plots of 9 m^2 (3 m × 3 m). Next to these mixture plots, we also set up 14 monoculture plots of 4 m^2 (2 m × 2 m) in which each species from our species pool

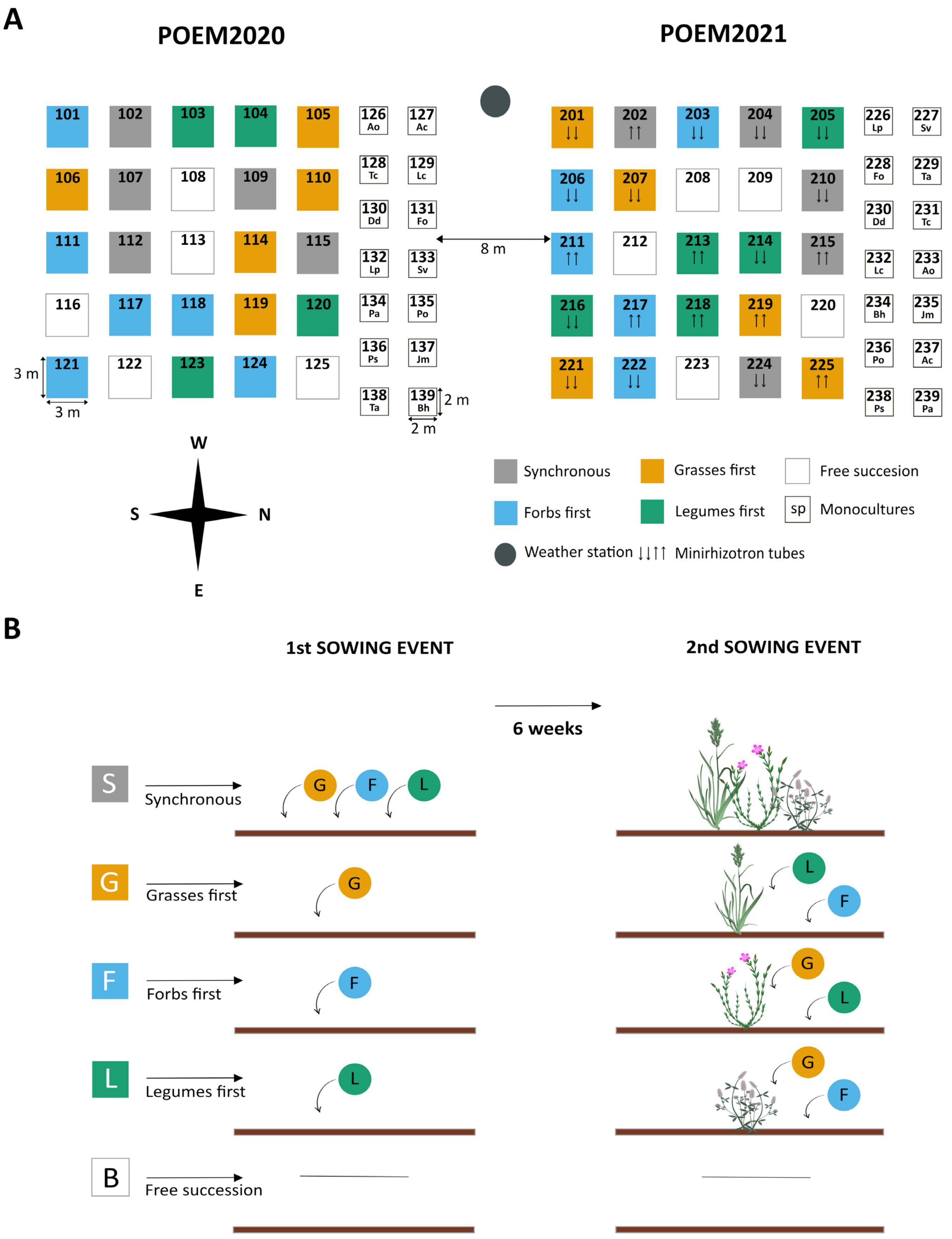

FIGURE 1 | Legend on next page.

FIGURE 1 | Overview of the POEM experiment. Panel A shows the experimental design for the first two sub-experiments (POEM2020 and POEM2021). Each sub-experiment was set up in a different year and consists of 25 mixture plots ($3 \times 3\,m^2$) and 14 monoculture plots ($2 \times 2\,m^2$). Plot ID numbers are shown inside the plots. Each PFG order of arrival scenario is represented by 5 replicates. Ac, *Agrostis capillaris*; Ao, *Anthoxanthum odoratum*; Bh, *Bromus hordeaceus*; Dd, *Dianthus deltoides*; Fo, *Festuca ovina*; Jm, *Jasione montana*; Lp, *Lathyrus pratensis*; Lc, *Lotus corniculatus*; Po, *Pilosella officinarum*; Ps, *Pimpinella saxifraga*; Pa, *Potentilla argentea*; Sv, *Silene vulgaris*; Ta, *Trifolium arvense*; Tc, *Trifolium campestre*. Panel B shows the PFG order of arrival scenarios tested in each sub-experiment. We tested five PFG order of arrival scenarios: (1) simultaneous sowing of forbs, grasses, and legumes at the first sowing event (synchronous, S), (2) forbs sown 6 weeks before grasses and legumes (F), (3) grasses sown 6 weeks before forbs and legumes (G), (4) legumes sown 6 weeks before forbs and grasses (L), and (5) no sowing of additional species (free succession, B).

grew on its own, and here the plots were regularly weeded. Monoculture plots (1 plot per species) were set up for every year of initiation (Figure 1A). We have explained how the monoculture plots were set up for the sake of completeness, but in this paper, only the data collected in mixture plots will be presented. Within each sub-experiment, experimental treatments and species identity were randomly assigned spatially to mixture and monoculture plots, respectively. Both PFG order of arrival (5 levels: S, F, G, L, B) and the year of initiation of the experiment (2 levels: POEM2020 and POEM2021) are considered fixed factors in the experiment.

Our experimental site has been equipped with a weather station (WS700-UMB, OTT HydroMet Fellbach GmbH, Fellbach, Germany) and additional sensors that continuously monitor air temperature (°C), air relative humidity (%), precipitation (mm), precipitation intensity (mm/h), wind speed (m/s), wind direction (°), atmospheric pressure (hPa), global radiation (W/m^2), photosynthetically active radiation ($\mu mol\,m^{-2}\,s^{-1}$), soil volumetric water content at 10, 20, 30, and 40 cm (%), and soil temperature at 5 cm (°C).

2.4 | Experimental Setup

Before starting each sub-experiment, the experimental area was harrowed, plots were marked and weeded, and large stones were removed by hand. The first sowing event took place on April 27, 2020 (for POEM2020) and April 13, 2021 (for POEM2021). The second sowing event took place 6 weeks later on June 8, 2020 (for POEM2020) and May 25, 2021 (for POEM2021) (Appendix S1). We chose this time of the year as we know from experience that grasslands in North Germany start their active growing phase in around early April.

Before starting each sub-experiment, the germination rate of each species was measured under controlled conditions. Mixture and monoculture plots were sown with 1000 viable seeds per m^2. Seed mixtures were prepared so that (1) all PFG had the same relative abundance and (2) all species within each PFG had the same relative abundance. A detailed description of the composition of the seed mixtures used for each sub-experiment is provided in Appendix S2 and S3. In the first sowing, the seeds were mixed with two cups of sand and spread evenly over the surface of the plots. A similar approach was used in the second sowing, but the seeds were sown from above, as we did not mow the plots before the second sowing, and we were careful not to disturb the plants growing in the plots. After starting each sub-experiment, we stopped weeding unsown species (i.e., species invading the plots or originating from the soil seedbank) because the longer term goal is to simulate ecological restoration where only one (or two) sowing events usually are used, and then the site is allowed to undergo natural assembly processes. Monoculture plots, however, were regularly weeded. The most common unsown species at our site were the annuals *Spergula arvensis* L., *Erodium cicutarium* (L.) L'Hér., *Anthemis arvensis* L., *Chenopodium album* L., *Fallopia convolvulus* (L.) Á. Löve, and the perennial *Rumex acetosella* L. The surroundings of the experiment and the paths between the plots were sown with the non-clonal grass *Festuca rubra* spp. *commutata* Gaudin. All plots were mown once a year in August.

2.5 | Installation of Minirhizotron Tubes

In order to be able to non-destructively monitor root development and distribution over time, we installed acrylic minirhizotron tubes in synchronous, forbs-first, grasses-first, and legumes-first plots of POEM2021. We did not install minirhizotron tubes in the first sub-experiment (POEM2020) because we did not have the capacity to monitor root development in two sub-experiments simultaneously. We chose to focus our efforts on regularly acquiring images at sufficient depth values in a single sub-experiment to be able to properly assess how the order of arrival of PFG affects root dynamics.

In POEM2021, minirhizotron tubes were installed in October 2020, i.e., 6 months before starting the second POEM sub-experiment. In each plot, two one-meter-long minirhizotron tubes (Vienna Scientific Instruments GmbH outer diameter: 60 mm; wall thickness: 3 mm) were installed at a 45° angle using a 58-mm diameter soil corer (Vienna Scientific Instruments GmbH). All minirhizotron tubes were closed on both sides and were installed as shown in Appendix S4, with the upper 21 cm of the tubes sticking out of the soil. The aboveground portion of the observation tubes was closed with a water- and light-tight plastic cap and covered with pipe insulation foam to exclude light and reduce thermal fluctuations. Within a plot, minirhizotron tubes were installed in the same direction (east–west or west–east), which was randomly selected for each plot. After the installation of minirhizotrons, the experimental area of POEM2021 was covered with a water-permeable ground sheet in order to avoid plant growth. This ground sheet was removed in April 2021, just before the first sowing event. A step-by-step description of the installation of minirhizotron tubes in POEM2021 is available in the video provided as Supporting Information (Appendix S5).

2.6 | Aboveground Data Collection

The structure of plant communities was assessed once a year by recording the total shoot dry weight of each individual species (sown and unsown species) located inside two randomly positioned 0.1 m^2 quadrats (20 cm × 50 cm). Four quadrats per plot were harvested at the end of the first growing season of POEM2020, but data showed that good estimates of community productivity can be achieved with only two quadrats (Appendix S6). These harvests were organized each year during the peak biomass production in June–July. At harvest, plants were cut 3 cm above the soil surface and sorted at the species level directly in the field. Shoot samples were dried in an oven at 60°C for at least 48 h and weighed on an analytical scale. For each plot, total aboveground productivity was calculated as the sum of the contributions of each individual species growing in the communities.

In order to get better estimates of the realized plant species richness inside the plots, the presence and absence of sown and unsown species were also monitored once a year, typically before collecting shoot biomass samples. These data were collected in all sub-experiments as of June 2021.

2.7 | Root Image Acquisition

High-resolution images of roots growing along the transparent minirhizotron tubes were acquired regularly using the VSI MS-190 manual camera (Vienna Scientific Instruments GmbH). Root images (2340 × 2400 pixels; resolution: 148 pixels/mm) were taken at 18 different depths, equally spaced from 1.4 cm to 49.5 cm deep (Appendix S4). When taking root images, the camera was always pointing upwards. Weather permitting, image acquisition was conducted twice a month from April 2021 to September 2021, once a month from October 2021 to March 2022, twice a month from April 2022 to September 2022, and once a month thereafter. Images were collected at 33 time points between April 2021 and June 2023 (3 growing seasons), which represent a total of 23,720 root images.

2.8 | Root Image Analysis

Root images were analyzed using an approach similar to (Alonso-Crespo et al. 2023). First, we used RootPainter (Version: 0.2.27) to train a convolutional neural network to detect roots in our images (Smith et al. 2022). A figure describing the procedure used to train a root segmentation model is provided as Supporting Information (Appendix S7). We first created two independent datasets of 1440 images (2 time points × 40 tubes × 18 depths). Each dataset consisted of all the images taken at two different time points, one year apart. We did this to ensure that each dataset included a wide variety of images (young/old roots, etc.). Then, we used RootPainter to randomly select two subregions (800 × 800 pixels) from each image of each dataset, thus creating two training datasets of 2880 images each. Each training dataset was then assigned to a different user with previous experience with image annotation and model training with RootPainter. Each user annotated at least 500 images of their training dataset (corrective annotations) and trained their model until predictions successfully identified most of the roots in training images. Both users used the same set of rules for annotating images. In particular, users aimed to train a model able to detect the centerline of living roots, thus avoiding dead roots, root edges, soil background, water droplets, and scratches at the surface of the minirhizotron tubes. This strategy proved useful in segmenting roots growing next to each other separately. Following this initial training procedure, the two users combined their training images and annotations and began training a third model, using the best of their two models as a starting point. This third model performed better than the models obtained by each user alone and was used to segment all root images in our dataset. Model training and image segmentation were done on a GPU node (NVIDIA Ampere A100 GPU with 40 GB memory) of a computer cluster available at the Leuphana University Lüneburg (Germany).

Segmented images were analyzed with RhizoVision Explorer 2.0.3 (Seethepalli et al. 2021) to estimate the total root length and the projected root surface area in each image. These parameters were then used to estimate the planar root length density (*pRLD*, total root length divided by image area, cm cm^{-2}) and the planar root surface density (*pRSD*, projected root surface area divided by image area, cm^2 cm^{-2}). Both *pRLD* and *pRSD* were highly correlated ($r = 0.96$, $p < 0.0001$). Because our image segmentation model was trained to detect the centerline of the roots, we probably underestimated *pRSD* and decided to focus on *pRLD* in this paper (but see Appendix S16 and S17 for results using *pRSD*).

2.9 | Data Analysis

Differences in plant community structure and community assembly trajectories were visualized using non-metric multidimensional scaling (NMDS). NMDS was performed with the metaMDS function of the R package vegan (Oksanen et al. 2022). We performed this analysis twice, on two different datasets: (1) species-specific plant biomass data (continuous data) measured for the three first growing seasons of POEM2020 and POEM2021, and (2) the presence (1) or absence (0) of species measured as of June 2021. We used the Bray–Curtis dissimilarity index for plant biomass data and the Jaccard dissimilarity index for presence/absence data. Before NMDS, biomass data were square-root transformed and standardized (Wisconsin double standardizations) prior to analysis, as these standardizations usually improve the results (Oksanen et al. 2022). In order to test for differences in composition between treatments, we conducted two permutational multivariate analyses of variance (PERMANOVA) using PFG order of arrival (categorical variable with 5 levels: S, F, G, L, B), year of initiation (categorical variable with 2 levels: POEM2020 and POEM2021), sampling year (categorical variable with 3 levels: year 1 to 3) and their interactions as fixed factors (Anderson 2001). F-statistics and P-values were computed based on 1000 permutations. All PERMANOVA models were fitted using the adonis2 function of the R package vegan (Oksanen et al. 2022). The same distance matrix was used for the NMDS and the PERMANOVA. Although distance-based multivariate analyses are known to confound location and dispersion effects, PERMANOVA has been shown to be largely insensitive to heterogeneity in multivariate dispersion between groups in the case of balanced designs (Anderson and Walsh 2013), which is the case in this study.

The effects of PFG order of arrival, year of initiation, and sampling year on plant diversity were analyzed using Hill numbers (Chao et al. 2014). For each combination of experiment, sampling year, and PFG order of arrival, we assessed how the effective number of plant species (D) changes with diversity order (q). Diversity order is a parameter used to adjust the sensitivity of D to the relative abundance of species in a community (Roswell et al. 2023). We calculated effective taxonomic diversity (D) of order q for S species using Equation (1a) ($q \geq 0$, $q \neq 1$) and 1b ($q=1$), where p_{ij} is the relative abundance of species i in plot j. This was done using the hillR R package (Li 2018). When $q=0$, all species are weighted equally, which is equivalent to species richness. Using larger values of q increases the weight of abundant species relative to rare ones. ${}^1D$ (i.e., effective diversity of order 1, referred to as the effective number of common species, or Hill Shannon) is equivalent to the exponential of Shannon entropy, while ${}^2D$ (i.e., effective diversity of order 2, referred to as the effective number of dominant species, or Hill Simpson) is equivalent to the inverse Simpson concentration (Roswell et al. 2023; Chao et al. 2024). In terms of interpretation, ${}^qD = x$ means that the diversity of order q of this assemblage is equivalent to an idealized assemblage consisting of x equally abundant species. The effects of PFG order of arrival, year of initiation, sampling year, and their interactions on plant diversity at $q=0$, $q=1$, and $q=2$ were analyzed using three separate generalized linear mixed-effect models. Models were fitted with the MASS (Venables and Ripley 2002) and lme4 (Bates et al. 2015) R packages using a quasipoisson ($q=0$) or a Gamma ($q>0$) distribution and a log-link function. Because data were collected in the same plots for 3 years, plot ID was used as a random effect in the models (random intercept). Post hoc tests were carried out using the emmeans R package (Lenth 2023).

$$ {}^qD_j = \left(\sum_{i=1}^{S} p_{ij}^{q} \right)^{1/(1-q)}, \quad q \geq 0, \quad q \neq 1 \tag{1a} $$

$$ {}^1D_j = \lim_{q \to 1} qD_j = \exp\left(- \sum_{i=1}^{S} p_{ij} \log p_{ij} \right), q = 1 \tag{1b} $$

The effects of PFG order of arrival, year of initiation, sampling year, and their interactions on total aboveground productivity (g/m^2) were analyzed using a generalized linear mixed-effect model. Plot ID was used as a random effect (random intercept). The model was fitted with the lme4 R package (Bates et al. 2015) using a Gamma distribution and a log-link function (strictly positive and continuous data). Post hoc tests were carried out using the emmeans R package (Lenth 2023).

As a proxy for root productivity in a plot at a given time point, we calculated the average planar root length density ($pRLD$) across 36 minirhizotron images (2 tubes/plot × 18 images/tube). To address the non-linear $pRLD$-time relationships for the different PFG order of arrival scenarios, we applied generalized additive models (GAMs) to assess changes in $pRLD$ over time. The fitted model included three components: (1) a fixed effect for PFG order of arrival, (2) a smooth function of time since the start of the experiment for each PFG order of arrival scenario (thin plate regression splines; the number of basic functions, k, was set to the number of time points in the dataset), and (3) a random-effect smooth for plot ID. This model was fitted with the mgcv R package (Wood 2017) using a tweedie family distribution with a log-link function. This distribution was chosen for its ability to model non-normally distributed continuous data with zero values, while ensuring that the predicted response remains positive (Zuur and Ieno 2021). We used the same approach to model the temporal evolution of $pRSD$ for each PFG order of arrival.

We assessed how PFG order of arrival affected the vertical distribution of roots in two complementary ways: (1) by modeling the temporal evolution of the average rooting depth of plant communities, and (2) by modeling the evolution of $pRLD$ as a function of time and soil depth. The same approach has been used for $pRSD$ data, so we focus solely on $pRLD$ in the following paragraphs.

At each time point, the mean rooting depth (MRD) in plot j was calculated using Equation (2), where d_{ij} is the soil depth at location i in plot j, and $pRLD_{ij}$ is the average $pRLD$ measured at location i in plot j. The temporal evolution of MRD was then modeled using a generalized additive model consisting of three components: (1) a fixed effect for PFG order of arrival, (2) a smooth function of time since the start of the experiment for each PFG order of arrival scenario (thin plate regression splines; the number of basis functions, k, was set to the number of time points in the dataset), and (3) a random effect smooth for plot ID. This model was fitted with the mgcv R package (Wood 2017) using a tweedie family distribution with a log-link function.

$$ MRD_j = \frac{\sum_{i=1}^{n} d_{ij} \times pRLD_{ij}}{\sum_{i=1}^{n} pRLD_{ij}} \tag{2} $$

Changes in $pRLD$ as a function of time and soil depth were modelled using a generalized additive model. The model included three components: (1) a fixed effect for PFG order of arrival, (2) a tensor product smooth function of time since the start of the experiment and soil depth for each PFG order of arrival scenario (cubic regression splines), which allows accounting for the interaction between time and soil depth, and (3) a random effect smooth for plot ID. This GAM was fitted with the mgcv R package (Wood 2017) using a tweedie family distribution with a log-link function.

Data analysis was performed in R version 4.2.3 (R Core Team 2023). In addition to the R packages mentioned above, the following R packages were used for data exploration, visualization, and analysis: R packages included in tidyverse (Wickham et al. 2019), car (Fox and Weisberg 2019), Hmisc (Harrell 2023), ggpubr (Kassambara 2023), gtools (Bolker et al. 2022), ggConvexHull (Martin 2017), MASS (Venables and Ripley 2002), and viridis (Garnier et al. 2021).

3 | Results

3.1 | Time Since Establishment Drives Plant Community Composition More Strongly Than PFG Order of Arrival and Year of Initiation

The effect of PFG order of arrival on species composition was similar across combinations of year of initiation and sampling year (i.e., no three-way interaction). This result was consistent

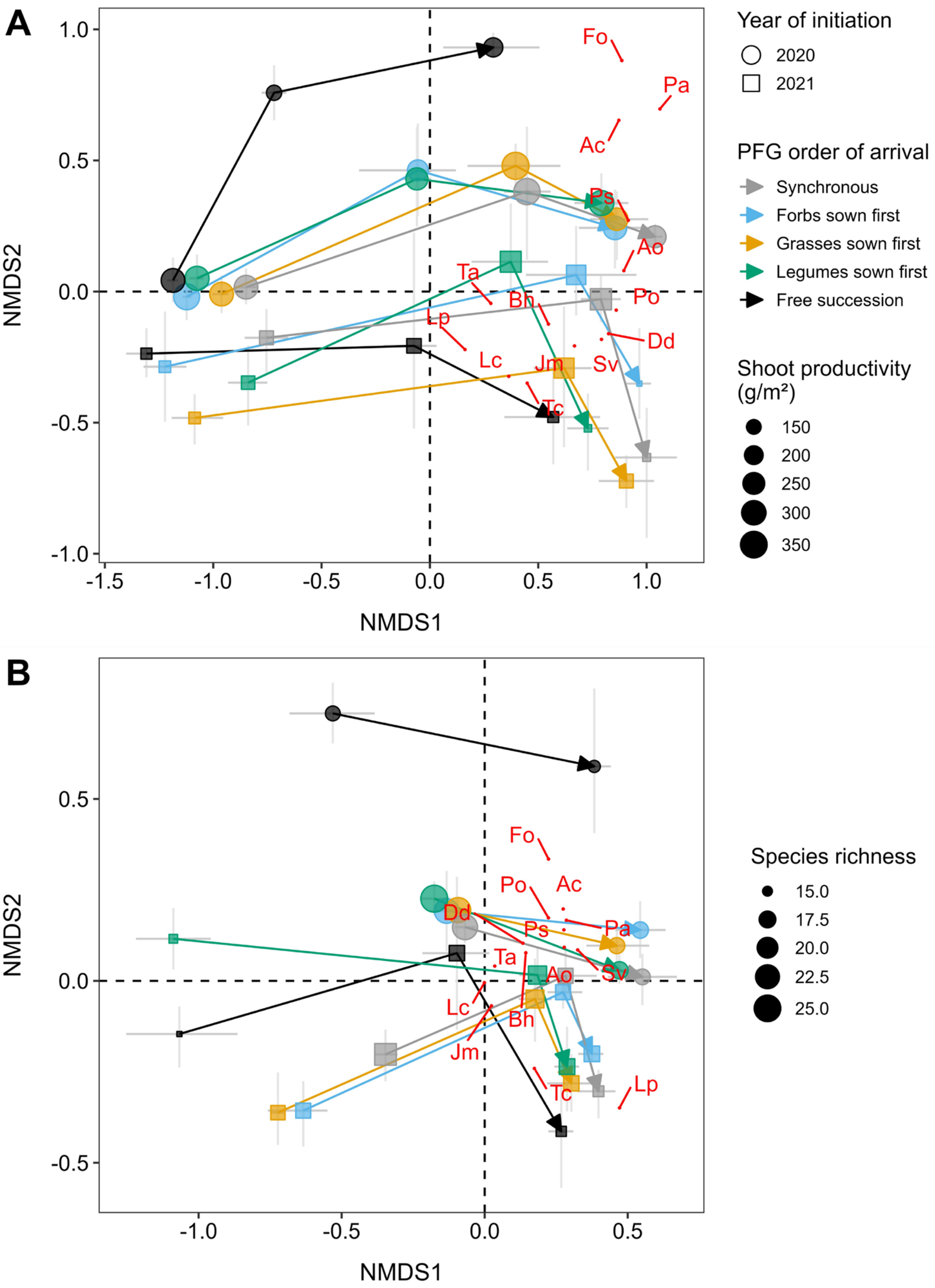

FIGURE 2 | Time since establishment drives plant community composition more strongly than PFG order of arrival and year of initiation. Panels A and B are non-metric multidimensional scaling (NMDS) plots showing dissimilarities in plant community composition observed after one, two, and three growing seasons between plots of two POEM sub-experiments (POEM2020 and POEM2021) in which PFG order of arrival was manipulated. Species-specific biomass data were used in panel A (stress: 0.166). Species presence/absence data were used in panel B (stress: 0.148; no data available for the first growing season of POEM2020). For each combination of year of initiation, sampling year, and PFG order of arrival, the centroid position and associated 95% confidence intervals computed using non-parametric bootstrap (grey segments) are shown ($n=5$). Species abbreviations: Ac, *Agrostis capillaris*; Ao, *Anthoxanthum odoratum*; Bh, *Bromus hordeaceus*; Dd, *Dianthus deltoides*; Fo, *Festuca ovina*; Jm, *Jasione montana*; Lp, *Lathyrus pratensis*; Lc, *Lotus corniculatus*; Po, *Pilosella officinarum*; Ps, *Pimpinella saxifraga*; Pa, *Potentilla argentea*; Sv, *Silene vulgaris*; Ta, *Trifolium arvense*; Tc, *Trifolium campestre*.

for both biomass (Figure 2A, $p=0.082$) and presence/absence data (Figure 2B, $p=0.966$). Using both data types, however, we found that the effect of PFG order of arrival on species composition was dependent on both the year of initiation of an experiment (two-way interaction between PFG order of arrival and year of initiation; Figure 2A, $p=0.003$; Figure 2B, $p=0.001$) and on sampling year (two-way interaction between PFG order of arrival and sampling year; Figure 2A, $p=0.001$; Figure 2B, $p=0.001$). Looking at main effects, we found that time since establishment explained much more variation in plant species composition (partial R^2: 37%–39%; Figure 2A, $p=0.001$; Figure 2B, $p=0.001$) than PFG order of arrival (partial R^2: 8%–10%; Figure 2A, $p=0.001$; Figure 2B, $p=0.001$) and the year of initiation of an experiment (partial R^2: 6%–8%;

Figure 2A, $p = 0.001$; Figure 2B, $p = 0.001$). This result was again consistent for both biomass and presence/absence data. Detailed PERMANOVA results are provided in Appendix S8 and S9.

Except for free succession plots, plant communities followed similar, but not identical, trajectories across PFG order of arrival scenarios within each sub-experiment, confirming that PFG arrival order plays a role, albeit a modest one, in modulating species composition (see Appendix S8 and S9 and next section on plant diversity). Plant communities in both experiments remained different over the 3-year study period, which highlights the important role of the year of initiation for community assembly in our POEM experiment (Figure 2, Appendix S8, S9).

During the first growing season of both sub-experiments, plant communities were strongly dominated by unsown annuals (*S. arvensis*, *E. cicutarium*, *A. arvensis*, *C. album*), which represented, on average, 97% and 93% of the total amount of biomass collected at harvest in POEM2020 and POEM2021, respectively. It was not until the second growing season that the sown species started to take over in the treatment plots, particularly in POEM2021 (Appendix S10). In the second growing season, sown species represented, on average, 77% and 93% of the harvested biomass in the first and second sub-experiments, respectively (Appendix S10). The relative proportion of sown species measured at the end of the third growing season reached very similar values (72% for POEM2020, 93% for POEM2021).

In both sub-experiments, forbs were most productive in plots where this PFG was given a head start, while grasses (especially *Bromus hordeaceus*) were usually more abundant in synchronous and grasses-first plots, particularly at the end of the second growing season (Appendix S11). Legumes, however, showed a more complicated pattern. Although *Trifolium arvense* established quite well in synchronous and legumes-first plots of POEM2021, legumes were not necessarily the most productive in plots where they were sown first (Appendix S11).

3.2 | Time Since Establishment, PFG Order of Arrival, and Year of Initiation Interactively Modulate Plant Diversity

Plant species richness was mainly affected by PFG order of arrival and time since establishment (Figure 3, Appendix S12). After one growing season, synchronous plots had on average seven species more than free succession plots in both sub-experiments. In synchronous communities of POEM2021, we also harvested on average four to six species more than in plots where one PFG was sown before the other two. After two and three growing seasons, the effect of PFG order of arrival on plant species richness was very similar in the two sub-experiments, although the results were only statistically significant for POEM2020. In the second year of POEM2020, plant species richness was higher in legume-first plots than in grasses-first and free succession plots. The same pattern was observed in the third growing season, although the difference in plant species richness between legumes-first and free succession plots was not statistically significant anymore (Figure 3).

The effect of PFG order of arrival on the effective number of common (i.e., Hill Shannon) and dominant species (i.e., Hill Simpson) in the plots depended on the combination of year of initiation and sampling year (three-way interaction for Hill Shannon: $p = 0.030$, Appendix S13; three-way interaction for Hill Simpson: $p = 0.053$, Appendix S14). In POEM2020, the effective number of common species was lower in grasses-first plots than in forbs-first and legumes-first plots, but only after two and three growing seasons. The same pattern was found for the effective number of dominant species in the second year of POEM2020. In the second and third years, the effective number of common and dominant species in synchronous plots of POEM2020 was as low as in grasses-first plots. These POEM2020 results highlight the overall negative effect that an early arrival of grasses had on plant diversity and community evenness in our experiment. This is mainly due to the strong dominance of the sown grass species *Bromus hordeaceus* (70%–81%) in synchronous and grasses-first plots after two growing seasons. We did not find this pattern in our second sub-experiment. In POEM2021, forbs-first plots had a lower effective number of common species than synchronous plots in Year 1 but had a number of common and dominant species in Year 3 that was greater than in grasses-first and free succession plots (Figure 3).

These results indicate that the PFG order of arrival modulates plant diversity, but that this effect evolves over time and depends on the year of initiation of an experiment.

3.3 | Year of Initiation, Not PFG Order of Arrival, Is the Main Driver of Aboveground Productivity

Across sub-experiments and sampling years, we never found any difference in standing shoot biomass production between plots in which the PFG order of arrival was explicitly manipulated (S, F, G and L plots), which strongly suggests that manipulating the order of arrival of forbs, grasses, and legumes only had a weak effect on aboveground productivity in our experimental system (Figure 4; see Appendix S15 for statistics). Standing shoot biomass differed between free succession plots and other PFG order of arrival scenarios, but only in the second growing season of both sub-experiments. In that year, plots in which all functional groups were sown simultaneously were, on average, 118% (POEM2020) and 72% (POEM2021) more productive than free succession plots. In the first sub-experiment (POEM2020), grasses-first plots were also 129% more productive than free succession plots in the second growing season. In the same year, legumes-first plots were 69% more productive than free successions in the second sub-experiment (POEM2021). The year of initiation of each experiment also had a major impact on the aboveground productivity of plant communities. On average, plots were 38% less productive in the sub-experiment set up in 2021 than in the sub-experiment set up in 2020 (Figure 4; Appendix S15).

3.4 | Root Productivity Was Weakly Affected by PFG Order of Arrival

Using the average planar root length density (*pRLD*, Figure 5A) and average planar root surface density (*pRSD*, Appendix S16)

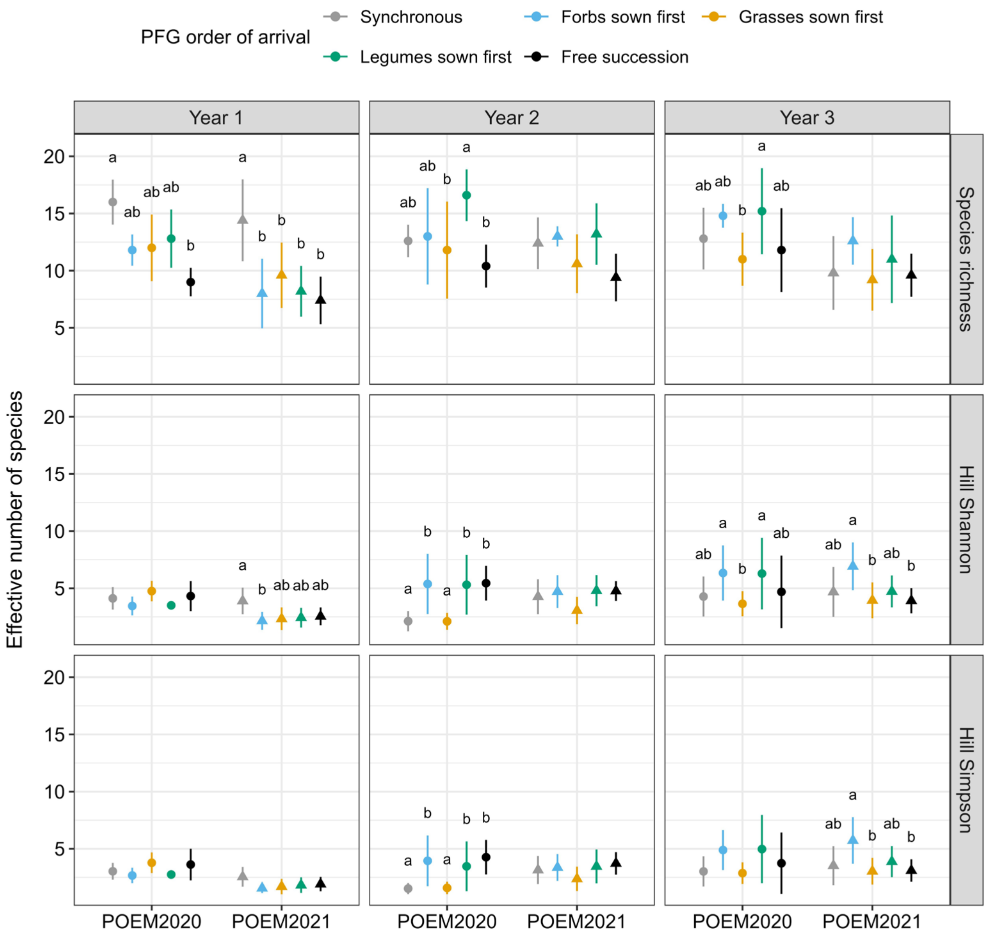

FIGURE 3 | Time since establishment, PFG order of arrival, and year of initiation interactively modulate plant species diversity. Each column represents a sampling year (Year 1–3), and each row represents a Hill diversity measure. For each combination of year of initiation, PFG of arrival, and sampling year, the mean value (closed dot) and 95% confidence interval computed using non-parametric bootstrap are shown ($n = 5$). S, synchronous sowing of forbs, grasses, and legumes; F, forbs sown first; G, grasses sown first; L, legumes sown first; B, free succession plots.

measured in a plot using minirhizotron images as proxies for standing root biomass production, we found that PFG order of arrival did not significantly affect belowground productivity over the 3-year study period (*pRLD*: $p = 0.275$; *pRSD*: $p = 0.166$). At peak root production (day 772 on May 25, 2023), however, the planar root length density measured in synchronous plots was, on average, 13%, 24%, and 19% greater than in plots where forbs, grasses, or legumes were sown first.

3.5 | Sowing Forbs or Legumes First Led to Deeper-Rooted Plant Communities

Although our results do not provide strong support for the existence of differences in root productivity between PFG order of arrival scenarios, they do show that manipulating the arrival order of grasses, forbs, and legumes had a strong impact on the vertical distribution of roots and the average rooting depth of plant communities (*pRLD*: $p = 0.005$; *pRSD*: $p = 0.004$). On average, forbs-first and legumes-first communities rooted 41% and 29% deeper than communities where grasses were sown first, respectively (Figures 5B and 6; F/G: $p = 0.0002$; G/L: $p = 0.0109$). We obtained similar results when the average rooting depth of plant communities was estimated using planar root surface density data (Appendix S17; F/G: $p = 0.0003$; G/L: $p = 0.0089$). We did not find strong evidence to support that the average rooting depth in synchronous communities was different from that in forbs-first, grasses-first, or legumes-first communities (Figures 5B and 6; S/F: $p = 0.076$; S/G: $p = 0.284$; S/L: $p = 0.542$). Although all PFG order of arrival scenarios led to the accumulation of roots ~15 cm below the soil surface, we also observed an accumulation of roots deeper into the soil (~40–45 cm), but mostly in plots where forbs or legumes were sown first (Figure 6; Appendix S17). This deeper root hotspot is particularly visible between 500 and 700 days after the start of POEM2021 (i.e., between September 2022 and March 2023).

4 | Discussion

Our results showed that the year of initiation had a strong impact on the aboveground productivity of plant communities but was a less important driver of species composition and diversity than time since establishment and PFG order of arrival. Although PFG order of arrival did not affect aboveground and belowground productivity, our results demonstrate that it can modulate the vertical distribution of roots at the community level, with grasses-first communities rooting more shallowly than forbs-first and legumes-first communities.

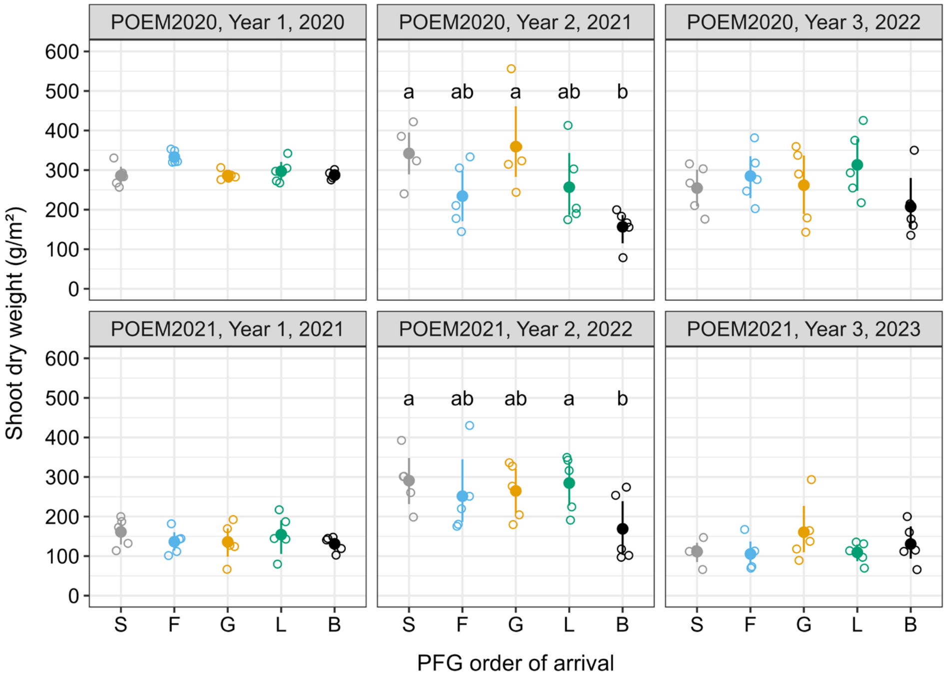

FIGURE 4 | Year of initiation, not PFG order of arrival, is the main driver of aboveground productivity. For each combination of year of initiation, PFG of arrival, and sampling year, the mean value (closed dot) and 95% confidence interval computed using non-parametric bootstrap are shown ($n = 5$). Open dots represent the observed shoot dry weight values, which are jittered horizontally to improve readability. S, synchronous sowing of forbs, grasses, and legumes; F, forbs sown first; G, grasses sown first; L, legumes sown first; B, free succession plots.

Contrary to our first hypothesis, we found that species composition was mainly driven by time since establishment and, to a smaller extent, PFG order of arrival and year of initiation. Plant diversity, however, was dependent on PFG order of arrival, year of initiation, and time since establishment.

We found that the effect of PFG order of arrival on plant species richness depended on the time since establishment. After one growing season, plant species richness was highest in plots where all species were sown simultaneously, while plots where one PFG was sown earlier than the others showed similar and intermediate levels of plant species richness. After two and three growing seasons, however, plots in which legumes were sown first were more species-rich than plots where grasses were sown first, with synchronous communities having intermediate levels of plant species richness. Previous studies from mesotrophic grasslands have shown that priority effects can affect community composition, with groups of species arriving first dominating communities (Körner et al. 2008; von Gillhaussen et al. 2014; Weidlich et al. 2017), which we did not find as strongly in our study after three growing seasons. Our results are in line with the findings of prairie grassland experiments manipulating grass and forb order of arrival (Werner et al. 2016), except that order of arrival effects are weaker in our system, probably because of our shorter sowing interval between early- and late-arriving species (6 weeks vs. 1 year) and the fact that we did not weed mixture plots after sowing.

Year effects have also been documented as a factor affecting community composition (Groves and Brudvig 2019; Werner et al. 2020). For example, in an experiment manipulating the timing of arrival of native and exotic grasses across three sites and 4 years, Stuble et al. (2017b) found strong evidence that the strength of priority effects exerted by native species after one growing season (when they were given a 2 week head start) was modulated by site location and year of initiation. This contrasts with the results of our POEM experiment obtained at the end of the first growing season, which can probably be explained by the fact that, unlike Stuble et al. (2017b), we did not weed unsown species in the mixture plots in order to let plant communities undergo natural assembly processes. In our POEM experiment, the sown species required time to establish, during which unsown weedy agricultural species established themselves (from the seedbank) in the first growing season of both sub-experiments. These unsown species initially established better than the species sown in the plots, but were strongly suppressed after mowing occurred, such that from the second growing season onwards, the sown species strongly dominated the plots. This effect was probably influenced by the timing of mowing in such dry acidic grasslands, which is later and less frequent (once versus twice per growing season) than in mesotrophic grasslands, as we know that mowing gives the perennial target species an advantage over the mainly annual ruderal species from the seedbank, especially in this field that was cropped for 200 years (Kirmer et al. 2018).

Overall, our findings are in line with expectations from ecological theory, that in more nutrient-poor dry acidic grasslands (e.g., our POEM experiment) competitive interactions (and hence asymmetric competition as a mechanism of priority effects within niche preemption) will be weaker (Chase 2003), but also suggest that longer sowing intervals may be needed to create larger priority effects (Werner et al. 2016). In both POEM sub-experiments, the grasses-first treatment generally exhibited lower taxonomic diversity over the three growing seasons, possibly due to the stronger competitive ability of grass species

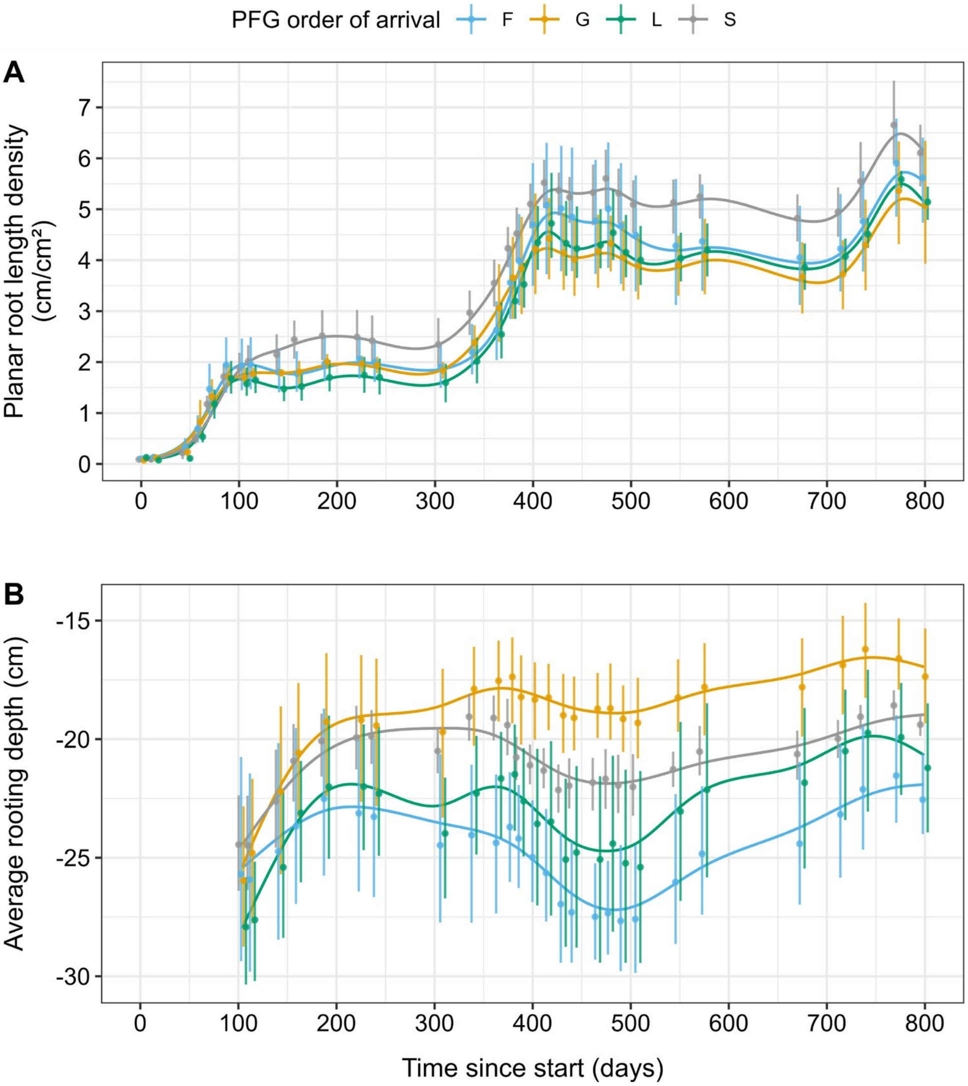

FIGURE 5 | Root productivity was weakly affected by PFG order of arrival, but sowing forbs or legumes first led to deeper-rooted plant communities. The figure shows the temporal evolution of the average planar root length density (*pRLD*, panel A) and average rooting depth of plant communities (panel B) measured in POEM2021 plots for each PFG order of arrival scenario using minirhizotrons. Points and error bars indicate mean values and 95% confidence intervals (non-parametric bootstrap) measured at 33 (for panel A) and 27 (for panel B) time points spread over the first 800 days of POEM2021, respectively. Continuous lines are predictions from generalized additive models. S, synchronous sowing of forbs, grasses, and legumes; F, forbs sown first; G, grasses sown first; L, legumes sown first.

when given an initial advantage (Linder et al. 2018), allowing them to outcompete other species and create stronger priority effects (Werner et al. 2016; Cadotte 2023).

Contrary to our second hypothesis, we did not find any strong effect of PFG order of arrival on standing shoot biomass production, with no difference in yield between synchronous, forbs-first, grasses-first, and legumes-first communities. However, we found that the year of initiation of an experiment was a stronger driver of aboveground community productivity, with plots sown in 2020 being, on average, more productive than those sown in 2021. The difference in productivity can probably be attributed to contrasting weather conditions during the first growing season of each sub-experiment (Bakker et al. 2003; Werner et al. 2020; Catano et al. 2023; Atkinson et al. 2023; Stuble et al. 2017a), but more work is needed to identify key weather variables driving this year effect. In contrast to previous studies on priority effects that manipulated PFG order of arrival in grassland ecosystems (Körner et al. 2008; von Gillhaussen et al. 2014; Weidlich et al. 2017), our results did not support any difference in productivity between PFG order of arrival scenarios. The absence of PFG order of arrival effects on aboveground productivity in our study could be due to (1) unfavorable conditions for the establishment of the sown species (related to weather conditions as well as initial dominance of unsown ruderal species from the seed bank) and (2) a possibly too short sowing interval for dry acidic grasslands.

Data from POEM2021, which is the only experiment so far equipped with minirhizotron tubes, did not support our third hypothesis. Using standing root length density (or root surface area) as a proxy, we did not find a strong effect of PFG order of arrival on root productivity (although synchronous plots tended to be more productive than the others). This contrasts

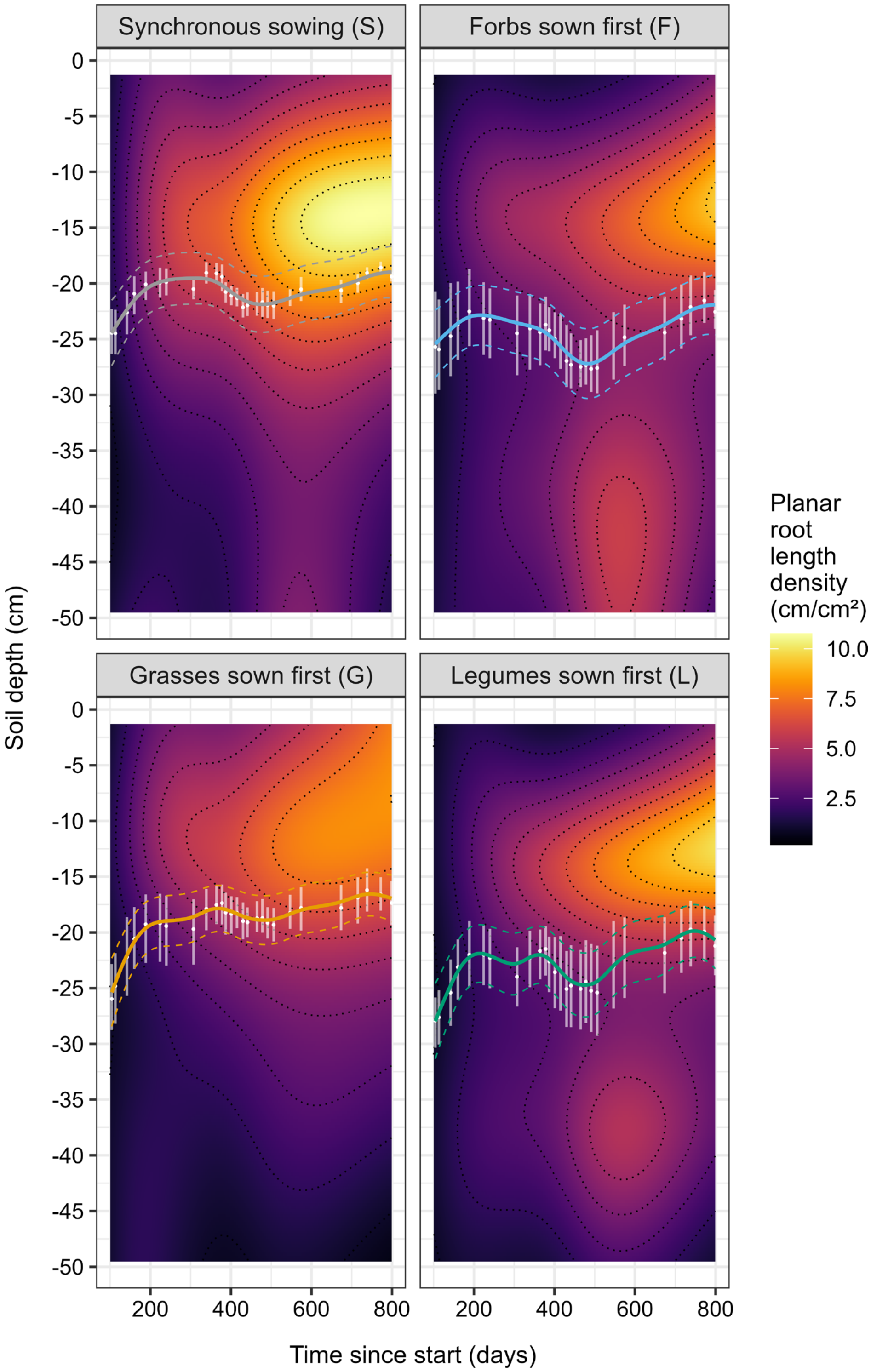

FIGURE 6 | Sowing forbs or legumes first led to deeper-rooted plant communities. The raster image in each panel shows predictions from a first generalized additive model of the planar root length density (*pRLD*) as a function of time (100–800 days) and soil depth (1.4–49.5 cm). Results are plotted separately for each PFG order of arrival scenario. On top of each raster, white dots and error bars indicate the mean values and 95% confidence intervals (non-parametric bootstrap) of the mean rooting depth (MRD) estimated from planar root length density data between days 100 and 800, respectively. Continuous lines are predicted MRD values from a second generalized additive model.

with findings from previous studies manipulating PFG order of arrival that measured root productivity in containers (Körner et al. 2008) or in the topsoil under field conditions (Weidlich et al. 2018). These studies have shown that communities where legumes were sown first have a lower root productivity or lower standing root length density in the topsoil (Körner et al. 2008; Weidlich et al. 2018). This discrepancy may be due to methodological differences; for example, in our study, root productivity was measured non-destructively over three years to a depth of around 50 cm, whereas in previous studies, root productivity was assessed destructively by extracting roots from soil cores.

In agreement with our fourth hypothesis, we found that PFG order of arrival had a strong effect on vertical root distribution at the community level. Indeed, we found that sowing legumes or forbs first led to communities rooting deeper than when grasses were sown first. Identical results were found when PFG order of arrival was manipulated in rhizoboxes under more controlled conditions (Alonso-Crespo et al. 2023). Considering that grassland species have different morphological and architectural characteristics, as well as different levels of root phenotypic plasticity in response to (a)biotic conditions (Herben et al. 2018; Bakker et al. 2019; Chen et al. 2020; Case et al. 2020; Lepik et al. 2021), slight differences in community composition following PFG order of arrival manipulation could lead to different patterns of root distribution. Given that grasses tend to root more superficially than forbs (Bakker et al. 2019, 2021; Chen et al. 2020), sowing grass species first may have increased root colonization and interspecific competition in the topsoil, which could have made it more difficult for later-arriving forbs and legumes to grow in deeper soil layers, thus making root distribution of the entire community more shallow. The fact that data from this field experiment and another controlled experiment in rhizoboxes (Alonso-Crespo et al. 2023) support a strong effect of PFG order of arrival on root distribution without affecting root productivity strongly suggests that previous observations that legumes-first communities have a lower standing root length density in the topsoil (Weidlich et al. 2018) may actually be due to differences in vertical root distribution, and not to differences in root productivity. To better understand the mechanisms behind plant order of arrival effects on root distribution and their ecological consequences for plant communities, additional work is needed to measure root distribution at the species level, for instance using molecular techniques based on DNA sequencing (Wagemaker et al. 2020). In addition, a forthcoming second subexperiment with minirhizotron measurements will allow us to see whether and how our root distribution findings are affected by the year of initiation.

5 | Conclusions and Outlook

We found that sowing legumes or forbs before the other plant functional groups caused deeper rooting than in other communities, whereas other factors such as the year of initiation or time since establishment had a stronger effect on aboveground community structure (composition and diversity) and functioning (aboveground biomass production). Given that our study accounted for priority effects on both above- and belowground dynamics, we provide experimental evidence that sowing legumes or forbs first can lead to communities associated with deeper roots. This, in turn, would allow communities to become more resistant to extreme weather events (e.g., drought) and potentially enhance soil carbon storage at depth. Implications for restoration efforts for grassland communities on sandy soils, however, would require further evidence from long-term (> 3 years) community trajectories, accounting for the fact that under nutrient-poor conditions, vegetation development takes time compared to communities that grow on sites with a better nutrient supply.

The disturbance regime at the POEM site is mowing (once a year), which differs from natural grassland sites that are often exposed to a more fire- or herbivory-driven disturbance regime. Both the disturbance intensity and frequency will be different in fire and herbivory-driven systems, and this may in turn influence the outcome of competition or facilitation between plant species. Thus, the potential for priority effects may be different in grasslands with another form of disturbance, highlighting the need to also assess the role of priority effects in fire- or herbivore-disturbed grasslands as well.

Author Contributions

V.M.T., B.M.D., and M.S. conceived the project, designed the experiment, and secured funding; I.M.A.C., B.M.D., T.N., and V.M.T. collected data; I.M.A.C. and B.M.D. analyzed root images; I.M.A.C., B.M.D., and A.F. analyzed data; I.M.A.C. produced the first draft of the manuscript, with support from B.M.D., A.F., and V.M.T. All authors contributed critically to the drafts and gave final approval for publication.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and R codes that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.10119982.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.