Causes and consequences of plant order of arrival on the structure and functioning of grassland communities

Inés María Alonso-Crespo

LEUPHANA
UNIVERSITÄT LÜNEBURG

LEUPHANA
UNIVERSITÄT LÜNEBURG

Causes and consequences of plant order of arrival on the structure and functioning of grassland communities

Der Fakultät Nachhaltigkeit
der Leuphana Universität Lüneburg zur Erlangung des Grades

Doktorin der Naturwissenschaften
– Dr. rer. nat. –

genehmigte Dissertation von
Inés María Alonso-Crespo
geboren am 18. Januar 1991 in Salamanca, Spain

Eingereicht am: 27.11.2023

Mündliche Verteidigung (Disputation) am: 19.04.2024

Erstbetreuerin und Erstgutachterin: Prof. Dr. Vicky Temperton, Leuphana Universität Lüneburg

Zweitbetreuer und Zweitgutachter: PD Dr. Andreas Fitchner, Leuphana Universität Lüneburg

Drittgutachterin: Prof. Dr. Tiffany Knight, Helmholtz Centre for Environmental Research – UFZ, Martin Luther University Halle-Wittenberg, German Center for Integrative Biodiversity Research (iDiv)

The individual contributions of the cumulative dissertation project are or will be published as follows:

- Alonso-Crespo, I. M., Temperton, V. M., Fichtner, A., Niemeyer T., Schloter M., & Delory, B. M. (2024) (*Submitted*) Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment. *Journal of ecology.* ID JEcol-2023-1025
- Alonso-Crespo, I. M., Weidlich, E. W., Temperton, V. M., & Delory, B. M. (2023). Assembly history modulates vertical root distribution in a grassland experiment. Oikos, 2023(1), e08886.https://doi.org/10.1111/oik.08886
- Alonso-Crespo, I. M., Temperton, V. M., Fichtner, A., Schloter M., Gschwendtner, S., & Delory, B. M. (*In prep.*). Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment

Copyright notice

Author’s address:
Leuphana University of Lüneburg, Institute of Ecology
Universitätsallee 1, 21335 Lüneburg, Germany
E-mail: ines.alonso_crespo@leuphana.de

"Those who dwell, as scientists or laymen, among the beauties and mysteries of the earth, are never alone or weary of life"

Rachel Carson. The sense of wonder

Table of Contents

SUMMARY

Grasslands are widely distributed ecosystems crucial for providing a variety of ecosystem services. Historically, they have been closely associated with humans. Some of the grasslands currently found in Europe are considered "semi-natural," as they have been shaped and managed by humans to support livelihoods through agriculture or livestock services. However, this strong human dependence has placed them at risk due to a growing divergence from traditional uses and management practices that originally fostered these species-rich ecosystems. These grasslands serve as habitats for a diverse range of species and provide important ecosystem services. Nonetheless, restoration efforts often face challenges and limitations. In the case of European grasslands, restoration initiatives must carefully navigate the trade-off between increasing and/or maintaining biodiversity and productivity simultaneously. It is needed to develop research focuses on long-term experiments to assess the factors that impact early stages of community assembly and, therefore, the outcomes of restoration practices.

Priority effects are the phenomena that occur when plant species arriving earlier in a community preempt and modify available niches, thereby influencing the establishment or development success of later-arriving species, either positively or negatively. The manipulation of the order of arrival of plant functional groups (PFG) has already been demonstrated to affect ecosystem functioning and community structure. However, it remains unclear to what extent the strength, trajectories, and persistence of priority effects can be influenced by the weather conditions experienced by ecological communities during the initiation year (referred to as "year effects"). Additionally, the role of the mechanisms of priority effects, niche preemption and niche modification, in the creation of these effects is still unknown.

In this thesis the results from three experiments are presented. These experiments were conducted to increase the knowledge of how priority and year effects can affect ecosystem functioning, above and belowground, and community composition and diversity in grassland ecosystems. In addition, the importance of the mechanisms of priority effects in their development was investigated.

The results showed that **1)** PFG order of arrival did not affect productivity above or belowground. **2)** However, aboveground productivity was predominantly driven by year effects. **3)** The time since establishment had a more significant impact on plant community composition compared to the order of arrival of plant functional groups and the year of initiation. **4)** The collective influence of these three factors affected plant diversity, with the effect of PFG order of arrival on plant species richness being contingent on the time since establishment and the year of initiation. **5)** PFG order of arrival notably influenced the vertical distribution of roots. Specifically, communities where grasses were introduced first exhibited shallower root systems compared to those where forbs or legumes were introduced first. **6)** Soil niche preemption and soil niche modification together are responsible for the creation of priority effects in dry acidic grasslands. Soil niche preemption seemed to be the primary driver, as soil niche modification alone does not yield significant effects on the development of plant functional.

Overall, these findings highlight the complex dynamics at play in grassland ecosystems. The order of arrival of plant functional groups combined with year effects, shapes community structure over time. This underscores the enduring influence of ecological timelines on the establishment of grassland communities. Moreover, the primary driver of aboveground productivity is the year effect, emphasizing the need to consider weather conditions in grassland restoration practices. Additionally, the order of arrival of plant functional groups significantly influences the vertical distribution of roots, suggesting possible implications for nutrient cycling and soil stability. Lastly, soil niche preemption and soil niche modification together triggers the creation of priority effects, with soil niche preemption being the predominant driving force in shaping specifically dry acidic grassland systems.

Keywords

Aboveground productivity, belowground productivity, community structure, ecosystem functioning, plant diversity, plant functional group order of arrival, plant-soil feedbacks, priority effects, soil niche preemption, soil niche modification, vertical root distribution.

ZUSAMMENFASSUNG

Das Grünland ist ein weit verbreitetes Ökosystem, das für die Erbringung einer Vielzahl von Ökosystemleistungen von entscheidender Bedeutung ist. Historisch gesehen sind sie eng mit dem Menschen verbunden. Einige der heute in Europa vorkommenden Grünlandflächen gelten als "halbnatürlich", da sie vom Menschen gestaltet und bewirtschaftet wurden, um den Lebensunterhalt durch Landwirtschaft oder Viehzucht zu sichern. Diese starke Verbindung zum Menschen hat das Grünland jedoch in Gefahr gebracht, da der Mensch zunehmend von den traditionellen Bewirtschaftungspraktiken abweicht, die ursprünglich diese artenreichen Ökosysteme förderten. Diese Grünlandflächen dienen als Lebensraum für eine Vielzahl von Arten und erbringen wichtige Ökosystemleistungen. Dennoch stoßen die Bemühungen zur Wiederherstellung häufig auf Herausforderungen und Grenzen. Im Falle des europäischen Grünlands müssen die Wiederherstellungsinitiativen sorgfältig zwischen der Erhöhung oder/und der Erhaltung der Artenvielfalt und der Produktivität abwägen. Die Forschung muss sich auf langfristige Experimente konzentrieren, um die Faktoren zu ermitteln, die die frühen Stadien der Gemeinschaftsbildung und damit die Ergebnisse der Wiederherstellungsbemühungen beeinflussen.

Prioritätseffekte sind Phänomene, die auftreten, wenn Pflanzenarten, die früher in einer ökologischen Gemeinschaft eintreffen, verfügbare Nischen präemtiv vorbelegen (preassign) und verändern und dadurch die Etablierung oder den Entwicklungserfolg später eintreffender Arten entweder positiv oder negativ beeinflussen. Es wurde bereits nachgewiesen, dass die Manipulation der Reihenfolge der Ankunft von Pflanzenfunktionsgruppen (PFG) die Funktionsweise von Ökosystemen und die Gemeinschaftsstruktur beeinflusst. Es ist jedoch noch unklar, inwieweit die Intensität, der Verlauf und die Dauer von Prioritätseffekten durch die Witterungsbedingungen beeinflusst werden können, denen ökologische Gemeinschaften im Jahr der Ansiedlung ausgesetzt sind (als "Jahreseffekte" bezeichnet). Darüber hinaus ist die Rolle der Mechanismen von Prioritätseffekten, Nischenpräemption und Nischenmodifikation bei der Entstehung dieser Effekte noch unbekannt.

In dieser Dissertation werden die Ergebnisse von drei Versuchen vorgestellt. Diese Experimente wurden durchgeführt, um das Wissen darüber zu erweitern, wie Prioritäts- und Jahreseffekte die Funktion von Ökosystemen, ober- und unterirdisch, sowie die Zusammensetzung und Vielfalt von Gemeinschaften in Grünlandökosystemen beeinflussen können. Darüber hinaus wurde die Bedeutung der Mechanismen von Prioritätseffekten für deren Entwicklung untersucht.

Die Ergebnisse zeigten, dass 1) die Reihenfolge der Ankunft der PFG keinen Einfluss auf die ober- oder unterirdische Produktivität hatte. 2) Die oberirdische Produktivität wurde hingegen hauptsächlich durch jährliche Effekte bestimmt. 3) Die Zeit seit der Etablierung hatte einen bedeutenderen Einfluss auf die Zusammensetzung der Pflanzengemeinschaft als die Reihenfolge der Ankunft der pflanzlichen Funktionsgruppen und das Jahr des Auftretens. 4) Der kollektive Einfluss dieser drei Faktoren wirkte sich auf die Pflanzenvielfalt aus, wobei der Effekt der Reihenfolge des Eintreffens der PFG auf den Artenreichtum der Pflanzen von der Zeit seit der Etablierung abhängig war und das Jahr des Beginns des Experiments. 5) Die Reihenfolge des Eintreffens von PFG auf den Wurzeln beeinflusste insbesondere die vertikale Verteilung der Wurzeln. Insbesondere hatten Gemeinschaften, in denen zuerst Gräser eingeführt wurden, flachere Wurzelsysteme als solche, in denen zuerst krautige Pflanzen oder Leguminosen eingeführt wurden. 6) Die Präemtion und die Modifikation der Bodennischen sind zusammen für die Entstehung von Prioritätseffekten in trockenen sauren Graslandschaften verantwortlich. Die Bodennischenpräemption scheint der primäre Faktor zu sein, da die Bodennischenmodifikation allein keine signifikanten Auswirkungen auf die Entwicklung der pflanzlichen Funktionsgruppen hat.

Insgesamt verdeutlichen diese Ergebnisse die komplexe Dynamik, die in Grünlandökosystemen im Spiel ist. Die Reihenfolge des Eintreffens von Pflanzenfunktionsgruppen in Verbindung mit Jahreseffekten prägt die Gemeinschaftsstruktur im Laufe der Zeit. Dies unterstreicht den dauerhaften Einfluss ökologischer Zeitpläne auf die Etablierung von Grünlandgemeinschaften. Darüber hinaus ist der Jahreseffekt der Hauptfaktor für die oberirdische Produktivität, was die Notwendigkeit unterstreicht, bei der Wiederherstellung von Grünland die Wetterbedingungen zu berücksichtigen. Zudem hat die Reihenfolge des Eintreffens von Pflanzenfunktionsgruppen einen erheblichen Einfluss auf die vertikale Verteilung der Wurzeln, was mögliche Auswirkungen auf den Nährstoffkreislauf und die Bodenstabilität erwarten lässt. Schließlich führen die Präemption und die Modifikation der Bodennischen zusammen zur Entstehung von Prioritätseffekten, wobei die Bodennischenpräemption die vorherrschende treibende Kraft bei der Gestaltung spezifischer trockener saurer Grünlandsysteme ist.

Schlüsselwörter

Ankunftsreihenfolge der Pflanzenfunktionsgruppen, Bodennischenmodifikation, Bodennischenpräemption, Gemeinschaftsstruktur, oberirdische Produktivität, Ökosystemfunktion, Pflanzen-Boden-Rückkopplung, Pflanzenvielfalt, Prioritätseffekte, unterirdische, Produktivität, vertikale Wurzelverteilung

Thesis framework

1. Thesis framework

1.1 Grassland ecosystems and the UN Decade of Restoration

Grasslands are global ecosystems present on every continent, excluding Antarctica, covering an area of 52.5 million km^2 (White et al., 2000). Many grasslands are closely linked to human activities (Habel et al., 2013), as they historically provide essential ecosystem services, such as agricultural services, water regulation, carbon storage, erosion control, climate mitigation, or pollination (Bengtsson et al., 2019). Some grasslands are considered "semi-natural" owing to significant human influence. These areas are often shaped and maintained through human activities, primarily grazing domestic livestock or haymaking, and would not exist without human intervention (Pärtel et al., 2005). Since the Anthropocene, these ecosystems became prevalent being widespread across Europe (Poschlod et al., 2009). Land use change, land-use intensification (i.e., increment in agricultural practices, fertilization, drainage, or increase in pasture intensity), and land-use abandonment are among the multiple causes leading to a decline in species-rich grassland (Gibson, 2009), resulting in losses in terms of functioning and biodiversity. In the specific case of European grasslands, the current distancing from traditional management and uses that originally led to the creation and maintenance of species-rich grasslands is the main reason for their threat. The mere act of abandonment results in degradation and loss of these grasslands.

Grassland ecosystems harbour high species richness and endemism (Buisson et al., 2019; Habel et al., 2013; Wilson et al., 2012), they are resilient ecosystems against extreme climate conditions or fire, and also play a role globally as belowground carbon sinks (Bai & Cotrufo, 2022; Dass et al., 2018). All in one gives them an intrinsic value for conservation. Due to the significant losses and challenges currently faced by grasslands, they have been identified as an important ecosystem that will require attention in the coming decades (Dudley et al., 2020). The United Nations Decade on Ecosystem Restoration seeks to protect and revive ecosystems globally in the next decade (2021-2030) including grasslands among the target ecosystems. With this aim, ten actions are listed in its strategy. One of these actions incentivizes investment in research that considers the complexity of restoration in the context of actual global change (*UN Decade on Restoration*).

Restoration practices do not always yield successful outcomes because of various interacting factors in natural systems. These factors include, for example, the selection of species mixes for restoration, biotic interactions among introduced species and species already present in the area, stochastic processes during community assembly, and a lack of information on the persistence of restoration efforts (Wilsey, 2020). To address these knowledge gaps and understand the factors that play an important role in restoration success, it is crucial to ensure successful restoration techniques (Temperton et al., 2016).

In the case of European grasslands, restoration practices should aim to strike a balance between increasing productivity and preserving biodiversity. Therefore, long-term experiments are needed to assess the persistence of practices that increase biodiversity and ecosystem functioning, while considering trade-offs. Additionally, these experiments should consider other environmental aspects that may reduce the success of these efforts, and the mechanisms underlying these processes. This thesis focuses on improving the understanding of *community assembly* in grassland plant communities, thus contributing to the knowledge base for successful grassland restoration. For this purpose, three different experiments, including a long-term study, were conducted. These experiments aimed to explore the consequences of the order of arrival of plant functional groups (i.e., *priority effects*) on community structure and ecosystem functioning above- and belowground in different grassland plant communities. They also aimed to disentangle the contribution of underlying mechanisms to these effects. Furthermore, it is also considered the interaction of the order of arrival of plant functional groups with weather conditions in the year of initiation of plant establishment.

1.2 Causes of priority effects

1.2.1 Plant-plant interactions and plant-soil feedbacks

Priority effects are defined as phenomena occurring in ecological communities where species arriving first can influence the performance of later-arriving species by occupying or changing the environment. (Fukami, 2015). Two main mechanisms have been described for the creation of priority effects: *niche preemption* and *niche modification* (Fukami, 2015). In *niche preemption*, early arriving species reduce the availability of resources in the niche (e.g., space, light, water, or nutrients) for later arriving species. In *niche modification*, early arriving species change the types of niche available for later-arrival species (e.g., microbial or chemical legacies). *Priority effects* can manifest as either facilitative or inhibitory. In facilitative scenarios, species arriving earlier exert a positive influence on those arriving later. Conversely, in inhibitory cases, species arriving earlier have a negative impact on those arriving later. When *priority effects* arise from *niche preemption*, early arriving species compete for available resources, often negatively affecting the establishment, survival, and growth of late-arriving species. Alternatively, when driven by *niche modification*, changes in niche types can be positive (facilitation), neutral, or negative (inhibition) (Fukami, 2015).

Priority effects mechanisms can alter how species interact. They can influence the strength of competition or *plant-soil feedbacks* (PSF) and may lead to facilitative interactions. On the one hand, negative priority effects can arise from the order of species arrival, influencing species interactions, such as competition, allelopathy, *plant-soil feedbacks*, such as soil sickness, or soil chemical legacies. Competition is defined as the interaction between individuals of the same or different species over a shared resource, which reduces the growth, survival, or reproduction of at least one of the individuals involved (Tansley, 1917; Tilman, 1982; Huston & Smith, 1987; Keddy & Shipley, 1989; Goldberg & Barton, 1992; Bengtsson et al., 1994; Goldberg, 1996). The order of arrival can confer an advantage that influences competition. Early arriving species can physically outcompete later-arriving species as they have more resources for development. Allelopathy is a negative interaction in which the establishment or growth of a species is delayed or impeded by the presence of chemicals released into the environment by some species (Rice, 2012). Early arriving species can release phytotoxins that delay or impede the establishment of later arriving species. Throughout *plant-soil feedbacks*, plants arriving earlier can alter the soil environment, microbiome, or nutrient availability for later arrival species (Bennett & Klironomos, 2019). Soil sickness is a type of PSF that refers to the negative conditions generated in the soil by plants, which can involve the reduction of available nutrients, increase in soil-borne pathogens, or release of phytotoxic compounds during decomposition (Cesarano et al., 2017). The strength of some *plant-soil feedback* interactions, such as *soil sickness*, can be affected by the identity of the early arriving species, thereby influencing the success of later arrivals. Soil chemical legacies refer to the long-lasting impact of chemicals released by plants that can affect subsequent generations of plants. These soil chemical legacies can create negative effects that affect plant development (Delory et al., 2021).

On the other hand, positive priority effects can arise from the order of species arrival, influencing species interactions such as facilitation, microclimate amelioration, nutrient enrichment, soil chemical legacies, and *plant-soil feedbacks*. Facilitation is a positive interaction that refers to the phenomenon in which one species positively affects another during the establishment, growth, or survival phases, specifically under stress conditions (Bertness & Callaway, 1994; Callaway, 1998; Brooker et al., 2008). The order of arrival of a specific species can facilitate the later arriving species. Direct facilitation can occur through microclimate amelioration, which is a positive interaction that occurs when a plant improves the conditions of a neighbouring plant by ameliorating environmental conditions (providing shade, improving soil conditions, or reducing competition for resources) (Brooker et al., 2008). Species arriving earlier with this capacity will increase the success of later-arriving species that require these specific conditions. Facilitation can also occur through nutrient enrichment by nitrogen-fixing legume species (Temperton et al., 2007; Wright et al., 2017). When these species arrive earlier, they provide nutrients to the later-arriving species, thereby facilitating their development. Soil chemical legacies of early arriving species can affect root development of later-arriving species (Delory et al., 2021). *Plant-soil feedbacks* can also have positive effects on subsequent species. For example, mycorrhizal-dependent plants grow in soils previously conditioned by compatible mycorrhizae (McMahen et al., 2022).

Box 1. To what extent do niche pre-emption and niche modification play a role in the creation of priority effects?

Species order of arrival can influence the strength of these interactions through *niche preemption* and *niche modification* mechanisms. Species arriving earlier can take advantage by exerting control through asymmetric competition (i.e., an unbalanced interaction where one species can outcompete the other), imposing specific soil feedbacks in the environment (which can either benefit or prejudice the development of later-arriving species), or facilitating the establishment of later-arriving species. However, the extent to which *priority effects* are driven by *niche preemption*, *niche modification*, or their combination remains unclear. One of the aims of thesis is to disentangle both mechanisms and reveal their contribution in the creation of *priority effects* through a *plant-soil feedback* experiment (Box 1).

1.3 Consequences of priority effects

1.3.1 Long-term consequences of priority effects on community assembly

Community assembly serves as the fundamental framework for comprehending the essential mechanisms that dictate a stable composition and population of species within a particular location, contingent upon specific environmental circumstances (Chase, 2003). During the last century, some approaches to explain the assembly of communities arose. The *deterministic theory* in the search for patterns governing species co-occurrence proposed a predetermined succession of stages based on associations dictated by physical and biotic factors such as species interactions (Clements, 1936; Cowles, 1899). The *stochastic theory* incorporated environmental factors, dispersal capacity, and species arrival order as shaping factors in community assembly (Gleason, 1926). In the 1960s, Lewontin, (1969) introduced the concept of alternative stable states, an intermediate point between deterministic and stochastic theories, emphasizing historical events as determinants. This notion was expanded by Sutherland, (1974) who identified species arrival order as a key historical event shaping community structure, which was first observed by Diamond, (1975) in bird populations. This theory proposes that communities may be predictable until a certain point, due to the random factors that affect the ecosystems, which can make them end in multiple stable stages.

The recognition of the impact of historical contingency has grown in recent years as it becomes more apparent. Historical contingency refers to past events that can significantly affect community assembly. These past events can be both abiotic (e.g., environmental conditions or disturbances) and biotic events (e.g., order of arrival of species), creating historical contingencies that affect the shape of ecological communities during the assembly process (Fukami, 2015).

In 2015, (Fukami, 2015) introduced a framework outlining community assembly outcomes driven by variations in species arrival history, making communities historically contingent. This phenomenon, known as *priority effects*, occurs when interactions between early- and late-arriving species are strong enough to influence species coexistence. Species arriving earlier can determine the success of later-arriving species in a particular environment, affecting their establishment and development in a positive or negative way (Fukami, 2015). This historical contingency, resulting from how species interact with each other depending on the order of arrival, can steer communities in different assembly trajectories, affecting composition and dynamics.

This framework identifies three long-term community-level consequences of historical contingency due to priority effects after a disturbance event such as frost, rainfall, soil disturbance, or fire (Crawley, 2004): *Alternative stable state*, *alternative transient states* and *compositional cycles*.

- *Alternative stable states* refer to the different community compositions that can be established depending on immigration history, even under the same environmental conditions and species pool. These stable states are resistant and can persist over time, unless they are heavily disturbed (Fukami & Nakajima, 2011).
- *Alternative transient states* refer to the dynamic states of a community that vary in structure and/or functioning in response to immigration history or other stochastic factors, despite having assembled under the same environmental conditions, having received the same set of species multiple times, and having undergone population dynamics over multiple generations of the

species involved. These transient states have not yet reached a stable state and can easily be disturbed, leading to changes in the community (Fukami & Nakajima, 2011).

- *Compositional cycles* refer to the states of a community in which a set of species occurs in a sequence that is repeated over time (Fukami, 2015; Morton & Law, 1997).

These states are long-term consequences of *priority effects*, and for them to occur, the species must colonize or attempt to colonize the community several times. They must also experience population dynamics over generations in the community (Fukami, 2015).

Additionally, the *dynamic environmental filter model* (Kelt et al., 1995) suggests that abiotic conditions at a site can influence and modulate the structure and functioning of ecosystems. This first happens by filtering the access of determined species to the system and second by impacting the growth, development, and survival of the organisms living on them. This conceptual model has commonly been used to explain the establishment and invasion of species during the assembly of ecological communities (Temperton et al., 2016).

Some of the abiotic conditions that can act as filters in communities are weather conditions. *Year effects* refer to the interannual variation that drives community assembly dynamics, with weather conditions being one of the most evident causes (Werner et al., 2020). The weather conditions that communities endure during assembly have a profound influence on the establishment, persistence, or species presence of the year of initiation, which can drive different assembly trajectories (Werner et al., 2020). Pitt & Heady, (1978) studied weather patterns and discovered that variations in precipitation regimes during different periods of the year determined the germination, productivity, or composition of crops and weeds, identifying grass or forbs years depending on precipitation conditions. Variations in precipitation and temperature or the interaction between weather conditions during the planting year can determine the outcomes of the community and therefore influence ecosystem dynamics (Bakker et al., 2003; MacDougall et al., 2008; Stuble et al., 2017.a; Groves & Brudvig, 2019; Werner et al., 2020), or functional and/or taxonomic composition (Atkinson et al., 2023; Catano et al., 2023). Snowpack, temperature, and low precipitation can affect seedling recruitment and survival (Werner et al., 2019), and specific weather conditions can define the invasion success of exotic species (Wainwright et al., 2012; Young et al., 2015).

So far, the interaction between *year effects* and *community assembly* has barely been explored due to the difficulty of replicating experiments with different years of initiation (Werner et al., 2020). However, few studies have considered the *year effects* in combination with other factors, including the manipulation of the order of arrival of species (Stuble et al., 2017.b; Werner et al., 2020). These studies revealed that *year effects* is a more important driver than the order of arrival of the species in the community assembly, even at different sowing intervals (Stuble et al., 2017.b; Werner et al., 2020). Nevertheless, these two studies focused on plant cover and community composition of grassland ecosystems.

A better understanding of the interaction between *year effects* and the manipulation of the order of arrival of species in the community is important to ensure effective restoration techniques in determinate ecosystems. This thesis focuses on the initial phases of *community assembly*. One of its aims was to explore the extent to which the order of arrival of species and their interaction with weather conditions during plant establishment can determine different community trajectories, shaping both aboveground and belowground functioning and community structure in dry grassland plant communities. In addition, it aims to investigate how long these divergences persist. To address these questions, the *POEM* field experiment was established (Box 2).

Box 2. To what extent the order of arrival of species and their interaction with the conditions of the year of initiation of the community can determine different community trajectories?

For how long are these divergences persistent?

1.3.2 Priority effect consequences on ecosystem functioning and community structure

In the realm of studying *priority effects*, two distinct approaches exist for manipulating the order of arrival. The traditional approach involves altering the sequence of arrival of individual species, either individually or in mixtures, considering only the unique characteristics of each species. This approach is commonly used in restoration ecology to reduce species invasions. The results of these studies shown that this manipulation can either prevent the establishment of invasive species or enhance natural biodiversity (Ejrnaes et al., 2006; Stevens & Fehmi, 2011; Martin & Wilsey, 2012; Vaughni & Young, 2015; Wilsey et al., 2015; Young et al., 2015; Stuble & Souza, 2016; Stuble & Young, 2020; Hess et al., 2020).

In contrast, a more contemporary approach focuses on manipulating the arrival of plant functional groups (PFG). This involves categorizing species based on their functional or morphological traits or their degree of phylogenetic relatedness (e.g., grasses, forbs, legumes), and introducing one group ahead of the others. This manipulation of the order of arrival of PFG is particularly relevant in systems such as European grasslands, where restoration actions must consider the trade-offs between increasing or maintaining productivity and preserving biodiversity. Knowledge from *Biodiversity-Ecosystem functioning* (BEF) experiments has highlighted the importance of the interactions between PFG in the community in terms of composition and functioning (Buchmann et al., 2018; Tilman et al., 1997; Weisser et al., 2017). Moreover, a systematic review (van der Plas, 2019) revealed that in many BEF studies, abiotic factors and the presence of certain PFG were stronger drivers of ecosystem functioning than biodiversity. Results from BEF studies indicated that increasing species diversity leads to higher productivity both aboveground and belowground. Furthermore, it affects other functions such as nutrient uptake, light penetration, carbon sequestration, nitrogen storage, soil fertility, or resistance to invasion (Hector et al., 1999; Isbell & Wilsey, 2011; Ruijven et al., 2003; Roscher et al., 2005; Steinbeiss et al., 2008; Jesch et al., 2018; Lambers et al., 2004; Marquard et al., 2009; Oelmann et al., 2011; Mueller et al., 2013; Ravenek et al., 2014; Meyer et al., 2016; Oram et al., 2018; Weisser et al., 2017). Nevertheless, BEF experiments, such as those mentioned earlier, do not manipulate the order of arrival of species or the PFG.

Other priority effects experiments have been conducted to predict community trajectories after a disturbance by monitoring different orders of arrival of plant species or PFG (Collinge & Ray, 2009; del Moral et al., 2009; Helsen et al., 2012; Sarneel et al., 2016). These studies found that community composition, species abundance, or growth strongly depend on the timing of species arrival in the systems. Recently, some studies have focused on the effect of manipulating the arrival of PFG on assembly in grassland communities (Körner et al., 2008; von Gillhaussen et al., 2014; Weidlich et al., 2017, 2018; Delory et al., 2019). These findings suggested that altering PFG immigration can affect community composition and functioning, leading to community assembly into different trajectories. This, in turn, could be an important tool for European grassland restoration, improving not only ecosystem functioning, but also habitat-specific diversity.

However, it remains unclear how strong or persistent these effects are depending on the context. The effect of manipulating the order of arrival has been documented to vary when interacting with other factors, such as sowing interval. (von Gillhaussen et al., 2014; Young et al., 2015), soil type (Weidlich et al., 2017; Young et al., 2015), soil properties (Sarneel et al., 2016), site age (Grman et al., 2013), or year effects (Stuble et al., 2017.b; Werner et al., 2020).

The order of arrival of species can affect community composition, diversity, and ecosystem functioning, and thus, community assembly trajectories. However, theses consequences seems to vary depending on the conditions. Moreover, their effects in the belowground part of the system remain limited. To explore the consequences of this manipulation on ecosystem functioning above- and belowground, this thesis contains two experiments with different grassland plant communities. The *POEM* field experiment, with dry grassland plant communities and the *Rhizobox* experiment, with mesic grassland plant communities under greenhouse conditions (Box 3)

Box 3. To what extent are the effects of manipulating the order of arrival of PFG significant depending on the system, and how persistent or predictable are they in combination with other factors?

What is the impact of manipulating the order of arrival of PFG on the belowground part of the systems?

1.4 General methodological foundations, objective, research questions and chapters

1.4.1 General methodological foundations

Priority effects occur when the species that arrive first in an ecological community influence the success of species that arrive later, either positively or negatively (Figure 1.1). Therefore, the main methodological foundation applied in the three experiments included in this thesis was the manipulation of the order of arrival of the three PFG. The selected plant functional groups were: Grasses, species belonging to the *Poaceae* family, Legumes, species members of *Fabaceae* family with the ability to fix atmospheric N_2, and Forbs, including species which do not belong to the grasses or legumes groups. By manipulating the arrival order of these three plant functional groups in two sowing events, arrival scenarios were created to study the different consequences of priority effects in grassland communities. The species pool is typical of European grassland ecosystems. The species composition of the *POEM* field experiment (*Chapter 1*) and the *plant–soil feedback* experiment (*Chapter 3*) consisted of species typically found in dry acidic grasslands. For the *Rhizobox* experiment (*Chapter 2*), the species composition was typical of mesic grasslands.

1.4.2 Objective and research questions

The overall objective of this thesis was to achieve a better understanding of the strength and mechanisms of *priority effects* and their consequences on community structure and ecosystem functioning, in grassland plant communities during the early stages of grasslands community assembly. Additionally, this thesis investigated whether the outcomes of *priority effects* are influenced by the weather conditions of the year of initiation of the plant communities (*year effects*) or whether these variations steer community dynamics. A comprehensive understanding of both the environment conditions and the processes contributing to stable natural ecosystems is crucial to accurately predict restoration outcomes, thereby enhancing ecosystem resilience through the improvement or generation of specific ecosystem functions and habitats. This is particularly important given the current global environmental changes that are expected to intensify in the coming years.

The following questions were addressed in this thesis:

I. Does the order of arrival of PFG significantly impact the above- and belowground productivity and/or community structure of dry acidic grassland plant communities? **(*Chapter 1)***

II. Does the year of initiation of plant communities (*year effects*) influence aboveground productivity and/or community structure and/or the outcomes of priority effects resulting from the manipulation of PFG order of arrival in dry acidic grasslands? **(*Chapter 1)***

III. Does the order of arrival of PFG significantly impact the vertical root distribution of dry acidic grassland plant communities? **(*Chapter 1)***

IV. Does the order of arrival of PFG significantly impact the above- and belowground productivity of mesic grassland plant communities? **(*Chapter 2)***

V. Does the order of arrival of PFG significantly impact the vertical root distribution of mesic grassland plant communities? **(*Chapter 2)***

VI. To what extent do soil niche preemption and soil niche modification or their interaction create priority effects in dry acidic grasslands? **(*Chapter 3)***

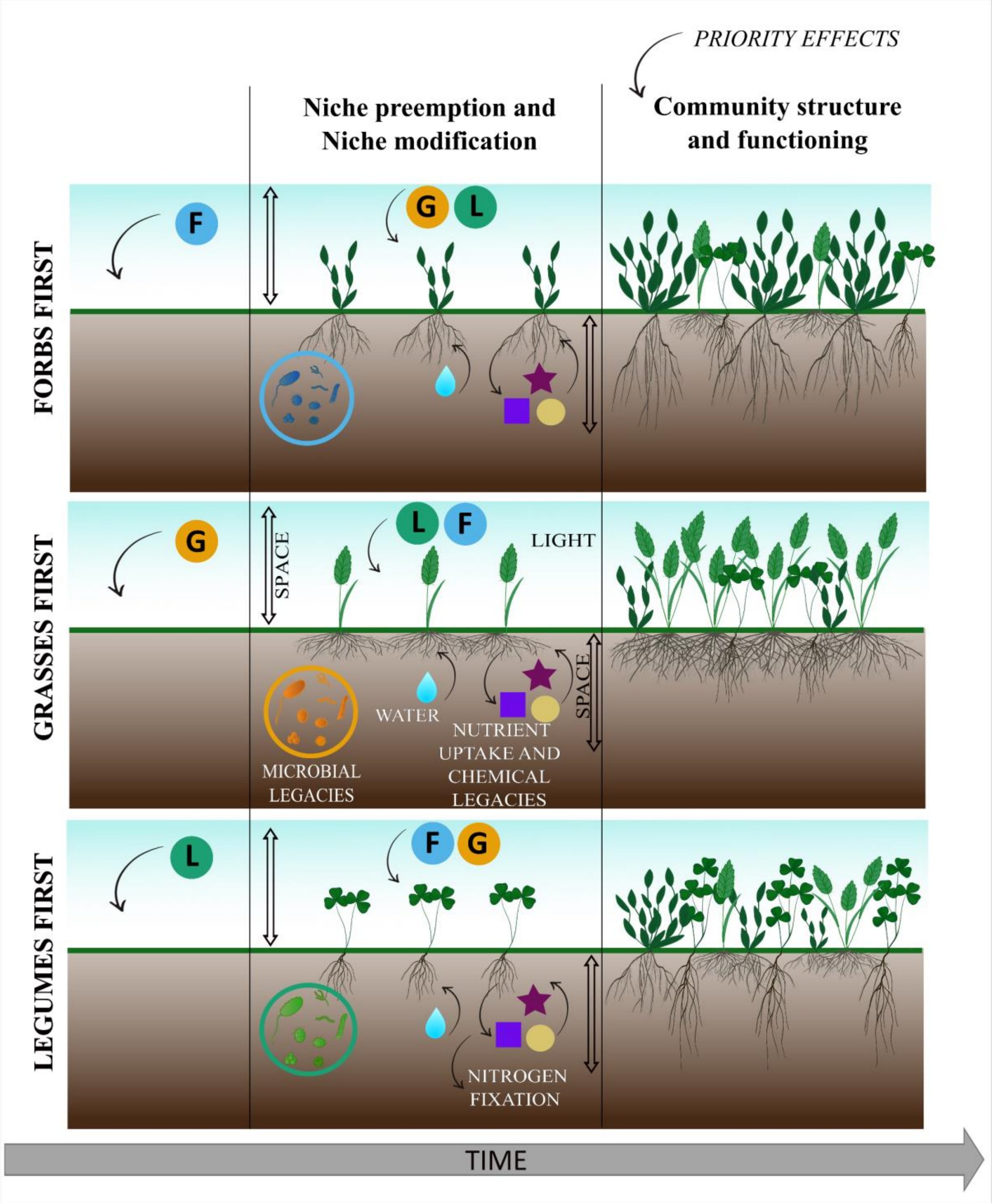


Figure 1.1. Conceptual framework of the thesis. Illustration of how the order of arrival of PFG can affect community structure and ecosystem functioning. Early arrival PFG preempt (e.g., nutrients, spaces, light, and water) and modify (e.g., via microbial or chemical legacies) the available niches, influencing the establishment and development success of the species from other PFG that arrive later. The order of arrival of species can determine plant-plant interactions due to plant-soil feedbacks or asymmetric competition. Different immigration scenarios can lead to diverse assembly trajectories derived from changes in the community structure and ecosystem functioning.

1.4.3 Thesis chapter's summary

- **Chapter 1. Exploring priority and year effects on plant diversity, productivity and verticalroot distribution: first insights from a grassland field experiment.**

The *POEM* (*P*ri*O*rity *E*ffect *M*echanisms) experiment.

This chapter focuses on how the order of arrival of PFG groups affects the community composition, diversity, and ecosystem functioning of dry acidic grassland plant communities above- and belowground in a field experiment. Additionally, it investigates how the weather conditions of the year of initiation (*year effects*) modulate the outcomes of *priority effects* resulting from the manipulation of the order of arrival of the PFG. The specific setup of this experiment allowed not only the testing of the effects of altering the order of arrival of the PFG but also how the year of initiation of the experiment modulates these effects aboveground.

By manipulating the order of arrival of three plant functional groups, grasses, legumes, and forbs, five treatments were created. This includes synchronous, where all the PFG arrived simultaneously in the 1st sowing event, grasses first, forbs first, or legumes first, where each respective group was the founder PFG sown in the 1st sowing, and the other two PFG were added 6 weeks later to the plots in the 2nd sowing. Finally, a treatment for free succession was established, in which any PFG was sown. Each treatment consisted of five replicates with a total of 25 plots per sub-experiment.

Two sub-experiments were initiated, replicating the same experimental design, in different and consecutive years (2020 and 2021) to compare the outputs of aboveground productivity and community structure, considering different weather conditions during the year of initiation. Belowground productivity and vertical root distribution were monitored only in the sub-experiments that started in 2021. In this sub-experiment, a minirhizotron imaging system was installed to register the root growth of the different treatments at various soil depths over a period of 800 days. Further information is detailed in *Chapter 1*, including the results section for the first three growing seasons of both sub-experiments, POEM2020 and POEM2021 (Figure 1.2).

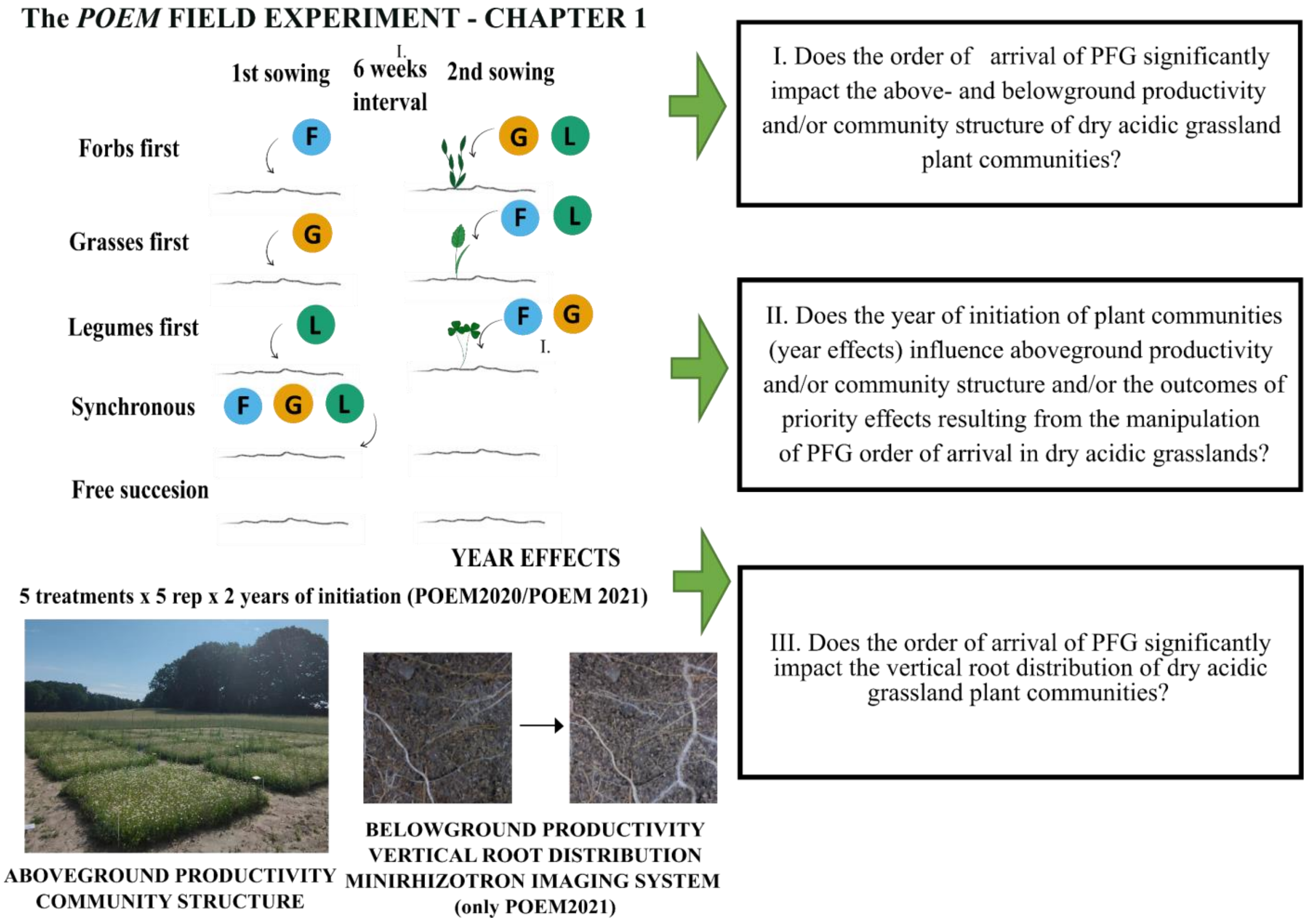


Figure 1.2 Methodological figure of *Chapter 1*: The *POEM* experiment. Illustration of the sowing treatments of the experiment, data acquisition and relation with the thesis research questions.

- **Chapter 2: Assembly history modulates vertical root distribution in a grassland experiment**

The *Rhizobox* experiment.

This chapter focuses on how the order of arrival of three plant functional groups affects above- and belowground productivity and the vertical distribution of roots of mesic grassland plant communities in a greenhouse experiment.

By manipulating the order of arrival of three plant functional groups, grasses, legumes and forbs, five treatments were created. This includes synchronous, where all the PFG arrived simultaneously in the 1st sowing event, grasses first, forbs first, or legumes first, where each respective group was the founder PFG sown in the 1st sowing, and the other two PFG were added 10 days later to the rhizoboxes in the 2nd sowing. Finally, a second synchronous treatment was also established, where all the PFG arrived simultaneously in the 2nd sowing event, allowing for a comparison with the plants sown in the second sowing. Each treatment consisted of seven replicates with a total of 35 rhizoboxes per sub-experiment. The use of rhizoboxes, a specific type of pot with a transparent front wall, allows image acquisition of root development over time. Further information is detailed in *Chapter 2*, including the results for aboveground and belowground productivity and vertical root distribution (Figure 1.3).

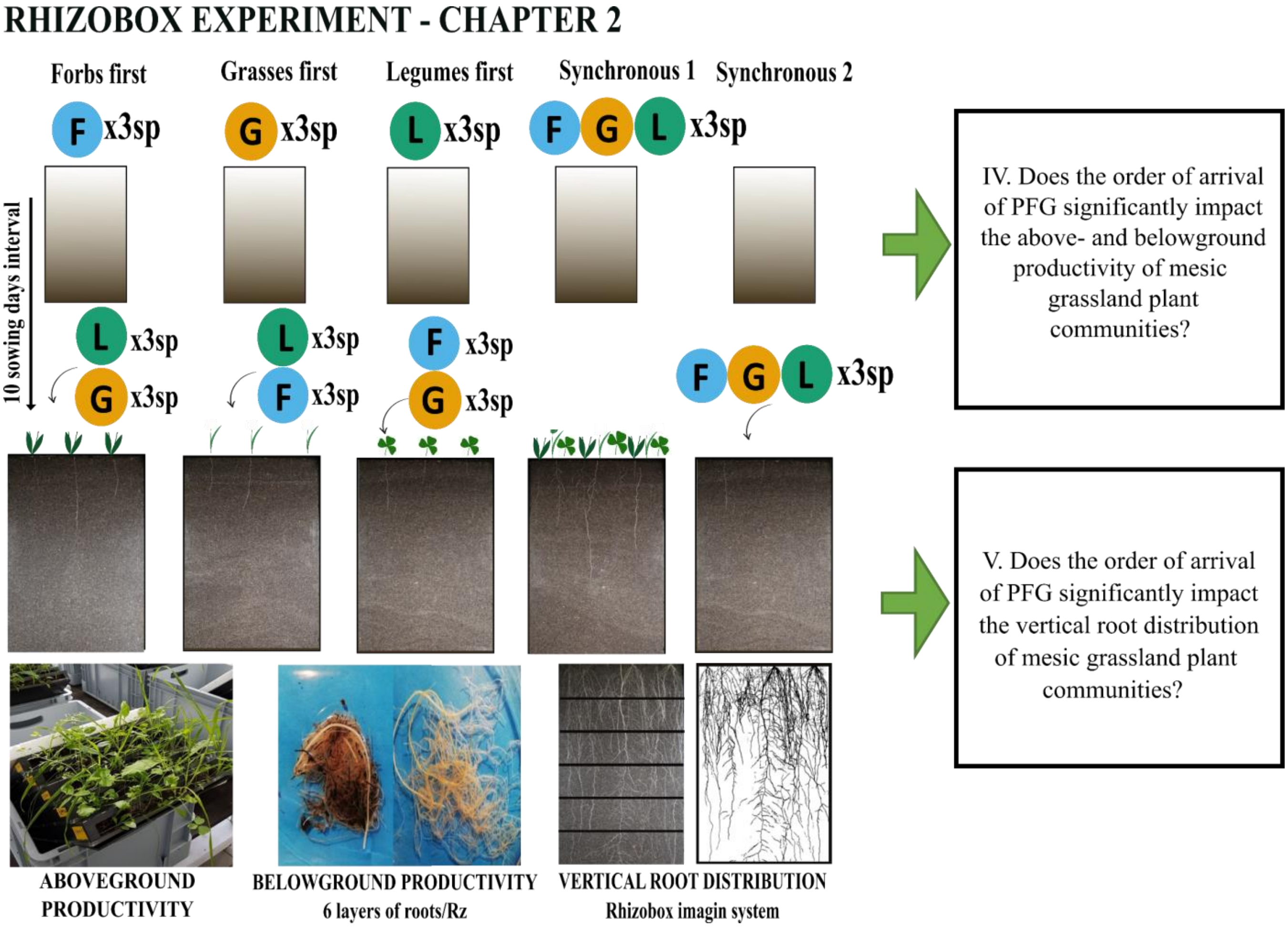


Figure 1.3: Methodological figure of *Chapter 2*: The *Rhizobox* experiment. Illustration of the sowing treatments of the experiment in the rhizoboxes, data acquisition and relation with the thesis research questions.

- **Chapter 3: Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment**

The *Plant-soil feedback* (PSF) experiment.

This chapter focuses in understanding the extent to which the *priority effects* mechanisms, *niche preemption*, and *niche modification*, play a role in the creation of priority effects. To assess the influence of these mechanisms, they were isolated within the soil context. *Soil niche modification* was identified through changes in microbial legacies, while *soil niche preemption* was associated with nutrient depletion. A *plant-soil feedback* experiment was conducted in two phases.

In the conditioning phase, four soil histories were established by sowing grasses, legumes, forbs, or a mixture of plant functional groups (one grass, one legume, and one forb). The conditioned soil from this phase was then utilized in the feedback phase. The feedback phase served as a response phase, exploring scenarios involving soil niche preemption + soil niche modification, using the conditioned soil from the conditioning phase, or soil niche modification alone, utilizing only an inoculum of the conditioned soil and sterilized soil. A control treatment was established, containing a mixture of sterilized soil and field inoculum, with no specific mechanisms at play. Individual plants belonging to forbs, grasses, or legumes were sown to determine their responses to different legacies and soil scenarios. Further information is detailed in *Chapter 3*, including the results for the aboveground and belowground productivity during the conditioning and feedback phases. (Figure 1.4)

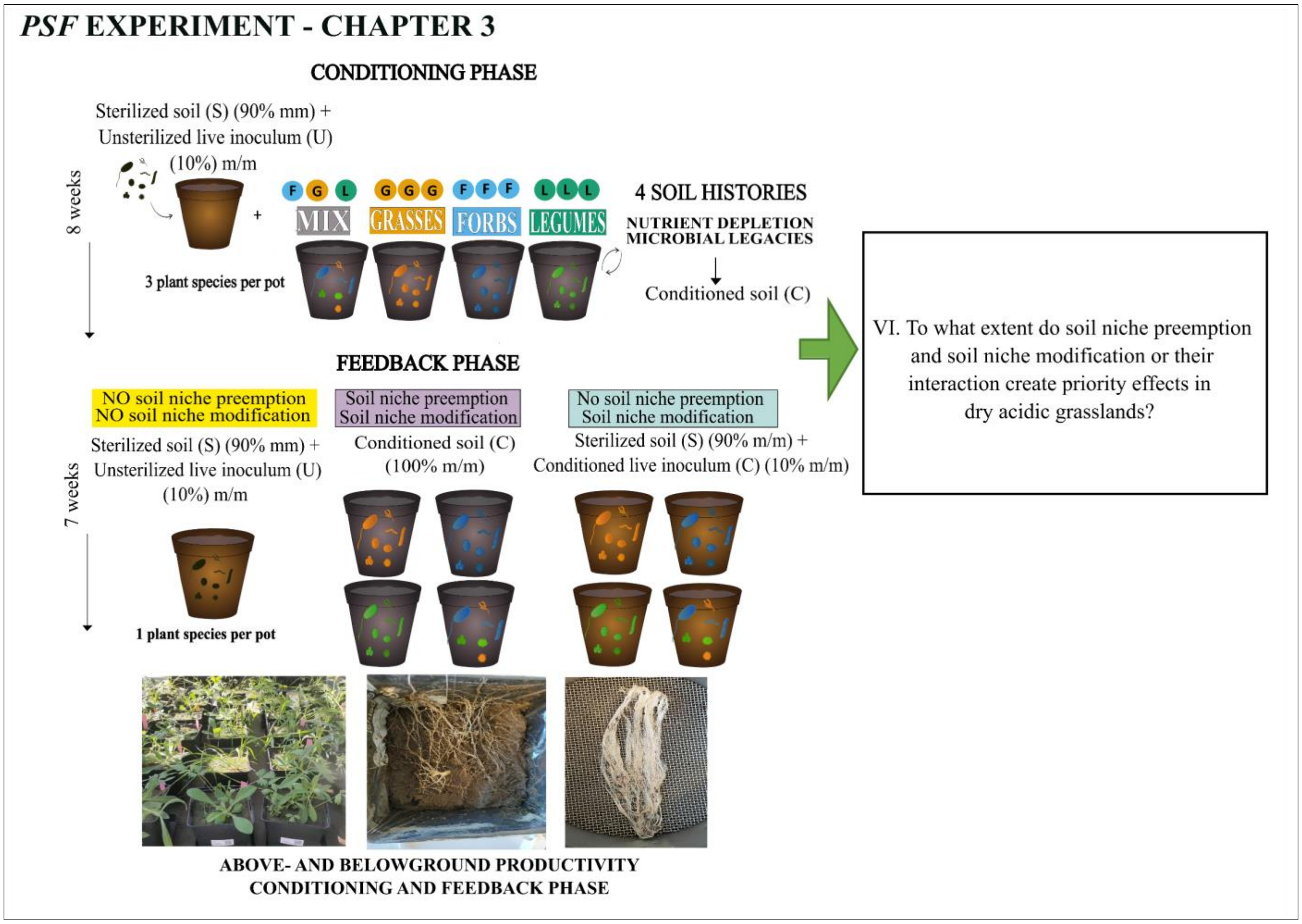


Figure 1.4: Methodological figure of *Chapter 3*: The *PSF* experiment. Illustration of the conditioning and the feedback phases, data acquisition and relation with the thesis research questions.

Chapter 1

Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment

Chapter 1

Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment

Submitted article: Journal of ecology. ID JEcol-2023-1025

Preprint available: https://biorxiv.org/cgi/content/short/2023.11.14.566982v1

Inés M. Alonso-Crespo, Vicky M. Temperton, Andreas Fichtner, Thomas Niemeyer, Michael Schloter Benjamin M. Delory

Abstract

1) 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.
2) Here we present the first results from a long-term field experiment (POEM: PriOrity Effects Mechanisms) 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 belowground. 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.
3) We found that time since establishment was a stronger driver of plant community composition than PFG order of arrival and year of initiation. These three factors interactively affected plant species diversity, with the effect of PFG order of arrival on plant species richness depending on time since establishment. Year of initiation, not PFG order of arrival, was the strongest driver of aboveground community productivity. Although we did not find any 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.
4) *Synthesis*: Our results demonstrate that plant order of arrival and year effects jointly affect plant diversity and species composition, with time since establishment also playing an important role. While year effects were more important than plant order of arrival in modulating aboveground biomass production in our nutrient-poor grassland, we showed that plant order of arrival can strongly affect the vertical distribution of roots, with communities in which forbs or legumes were sown first rooting deeper than grasses-first communities. These results suggest that a deeper understanding of priority and year effects is needed to better predict restoration outcomes.

Keywords: plant order of arrival, community assembly, vertical root distribution, species coexistance, field experiment, grassland restoration, productivity, community structure, plant diversity

Introduction

Grasslands are a dominant land use type all across the globe. Their conservation and restoration are critical to combat both the biodiversity and climate crises. In the UN Decade on Ecosystem Restoration (2021-2030), the pivotal role that species-rich grasslands could potentially play in both ensuring resilience in the face of extreme weather events, storing belowground carbon (Dass et al., 2018) as well as swiftly restoring biodiversity (Staude et al., 2023; Temperton et al., 2019) is gaining traction. Against this backdrop, there is increasing interest in implementing effective multifunctional grasslands into existing landscapes (Temperton et al., 2019; Wilsey, 2020). Until recently, however, species-rich grassland restoration was mainly focused on creating as biodiverse grasslands as possible, with aspects of ecosystem functioning (such as productivity, nutrient cycling or carbon storage) having only attracted concerted attention within more academic biodiversity-ecosystem functioning (BEF) experiments (Lambers et al., 2004; Weisser et al., 2017). Thus, we still lack a comprehensive understanding of how to restore grasslands to promote habitat-specific plant diversity and enhance ecosystem functioning at the same time.

Grassland 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 functional group establish first at a site after disturbance can alter not only plant diversity but also ecosystem productivity (Delory et al., 2019; Körner et al., 2008; von Gillhaussen et al., 2014; Weidlich et al., 2017). Such so-called priority effect approaches, whereby arrival order or initial relative abundance of species can significantly influence further community assembly, community structure and composition and/or ecosystem functioning (Drake, 1991; Fukami, 2015) are well known within the field of ecological restoration (Funk et al., 2008). Many priority effect studies have focused on reducing the performance of unwanted invasive species by sowing target species first to keep the invader out (Hess et al., 2019; Martin & Wilsey, 2012; Yannelli et al., 2020). However, the key limitation for grassland restoration is not invasive species, but a need for restoring diversity and aiming for higher productivity, resilience to drought or sequestering carbon in soils (Lyons et al., 2023). Sowing certain species or PFG before others may induce desired trajectories that create the kind of multifunctional outcomes we are currently looking for (Weidlich et al., 2021).

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 rest often having higher aboveground productivity, but lower root biomass in the topsoil. In a controlled experiment, 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). 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.

We expect stronger priority effects in ecosystems with higher availability of resources (Chase, 2003), since in more nutrient-rich sites competitive species may be better able to preempt resources and thus affect later arriving species through asymmetric competition, creating stronger priority effects. Conversely, if such priority effects include facilitative interactions, this may lead to more ecologically even communities that can create both plant diversity as well as perhaps resilience, better carbon storage and increased hay productivity (as a motivation to farmers to maintain such grasslands). Previous PFG priority studies have generally focused on more mesotrophic grassland settings (Weidlich et al., 2017), whereas the current study examines whether we can find above and belowground priority effects in a low nutrient dry acidic grassland.

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) possibly since it requires a huge effort to set up the same experiment repeatedly over time (Stuble et al., 2017.b). Evidence for year effects has been hard to extract from studies due to correlational considerations and confounding factors (Groves et al., 2020; MacDougall et al., 2008), but (Stuble et al., 2017.a) studied the effect of year of initiation and site on restoration outcomes and found both strong site and year effects on community composition. In another study, (Stuble et al., 2017.b) manipulated the timing of arrival of native and exotic grasses in Californian grasslands across different sites and years, and found that the strength of priority effects was strongly dependent on the location of the experimental site and the year in which an experiment was initiated. A study by (Groves & Brudvig, 2019) where year of initiation and precipitation were manipulated underlined that year effects can occur even if precipitation is held constant, suggesting an important role for environmental variables other than precipitation in driving year effects during restoration. (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 priority (sowing interval) and site treatments. 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 (Atkinson et al., 2023; Catano et al., 2023; Werner et al., 2020). To better predict restoration outcomes, it is necessary to delve deeper into the ecological mechanisms underlying year effects and their context-dependence.(Atkinson et al., 2023; Catano et al., 2023; Groves & Brudvig, 2019; MacDougall et al., 2008)

Although it has been shown that PFG order of arrival can affect community structure, above and belowground productivity, and root distribution in the soil (Alonso-Crespo et al., 2023; Körner et al., 2008; von Gillhaussen et al., 2014; Weidlich et al., 2017, 2018), 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 are a consequence of changes in the total root productivity 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.

Material and Methods

Study site

The POEM experiment is located on a former arable land owned by a local conservation organisation (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, 2020-2023). The experiment was set up on a soil that is appropriate for establishing dry acidic grassland communities (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 our experimental area: *Trifolium repens*, *Trifolium pratense*, *Trifolium incarnatum*, *Lolium perenne*, Festulolium (*Festuca* sp. × *Lolium* sp.) and *Secale cereale*.

Species pool and classification into plant functional groups

A total of fourteen 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* L., *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).

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 aboveground and belowground structure and functioning of dry grassland plant communities.

Year of initiation effects are tested by setting up the same experiment at the same site, but in a separate block, in four different years. Here, we use the results obtained for the first two sub-experiments set up in 2020 (referred to as POEM2020) and 2021 (referred to as POEM2021), respectively. Two additional sub-experiments will be set up in the coming years.

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 six weeks before grasses and legumes (F), (3) grasses sown six weeks before forbs and legumes (G), (4) legumes sown six weeks before forbs and grasses (L), and (5) no sowing of additional species (free succession, B) (Figure 1). 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 grew on its own and here the plots were regularly weeded. New monoculture plots were set up for every year of initiation. 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 that continuously monitors 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) (Figure S1.A).

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 six weeks later on June 8, 2020 (for POEM2020) and May 25, 2021 (for POEM2021) (Figure S1.B). 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 m². 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 Tables S1 and S2. 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 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.

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 this in October 2020, i.e. six months before starting the second POEM sub-experiment. In each plot, two one-metre 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 Figure S2, 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. Between October 2020 and April 2021, the experimental area of POEM2021 was covered with a water-permeable ground sheet in order to avoid plant growth. A step-by-step description of the installation of minirhizotron tubes in POEM2021 is available in the video provided as supplementary material.

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 m² 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. These harvests were organised 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 realised plant species richness inside the plots, the presence and absence of sown and unsown species was also monitored once a year, typically before collecting shoot biomass samples. These data were collected in all sub-experiments as of June 2021.

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 (Figure S2). 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 represents a total of 23,720 root images.

Root image analysis

Root images were analysed 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). To do this, we first created two independent datasets of 1,440 images. 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. Each dataset consisted of images from different time points. Then, we used RootPainter to randomly select two subregions (800×800 pixels) from each image of each dataset, thus creating two training datasets of 2,880 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 analysed 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 supplementary information for results using *pRSD*).

Data analysis

Differences in plant community structure and community assembly trajectories were visualised 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) species presence/absence (binary data) 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, square root transformation

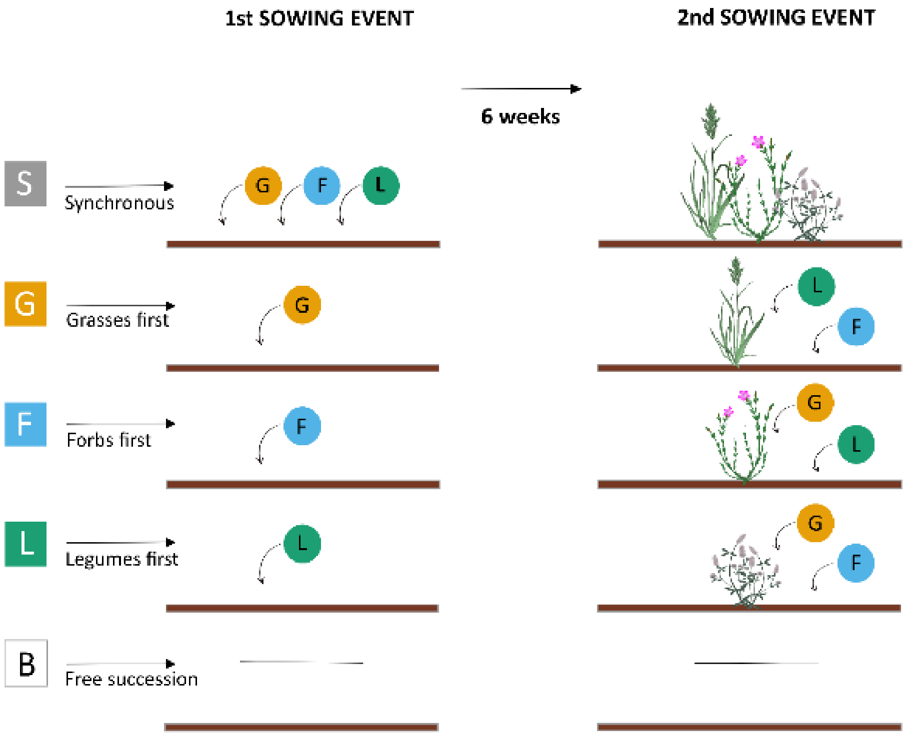


Figure 1. PFG order of arrival scenarios tested in the first two POEM sub-experiments. We tested five PFG order of arrival scenarios: (1) simultaneous sowing of forbs, grasses and legumes at the first sowing event (synchronous, S), (2) forbs sown six weeks before grasses and legumes (F), (3) grasses sown six weeks before forbs and legumes (G), (4) legumes sown six weeks before forbs and grasses (L), and (5) no sowing of additional species (free succession, B).

and Wisconsin double standardisation were applied to plant biomass data. 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 (Warton, Wright & Wang, 2012), PERMANOVA has been shown to be largely insensitive to heterogeneity in multivariate dispersion between groups in the case of balanced designs (Anderson & 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 analysed at the alpha level using Hill numbers (Chao et al., 2014). For each combination of experiment, sampling year and PFG order of arrival, we constructed diversity profile plots to visualise 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. We calculated effective taxonomic diversity (D) of order q for S species using Equations 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. ${}^{1}D$ (i.e., effective diversity of order 1) is equivalent to the exponential of Shannon entropy, while ${}^{2}D$ (i.e., effective diversity of order 2) is equivalent to the inverse Simpson concentration. In terms of interpretation, ${}^{q}D = x$ means that the diversity of order q of this assemblage is equivalent to an idealised 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 analysed using three separate generalised linear mixed-effect models. Each model was fitted with the lme4 R package (Bates et al., 2015) using a Gamma distribution and a log-link function. Because data were collected in the same plots for three years, plot ID was used as a random effect in the model (random intercept). Post-hoc tests were carried out using the emmeans R package (Lenth, 2023).

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

Equation 1a

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

Equation 1b

The effects of PFG order of arrival, year of initiation, sampling year and their interactions on total aboveground productivity (g/m²) were analysed using a generalised 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. 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). We then modelled the temporal evolution of *pRLD* for each PFG order of arrival using a generalised additive model (GAM). The 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 (thin plate regression spline), conditioned by PFG order of arrival (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. 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 modelling the temporal evolution of the average rooting depth of plant communities, and (2) by modelling 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 modelled using a generalised 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 (thin plate regression spline), conditioned by PFG order of arrival (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.

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

Changes in *pRLD* as a function of time and soil depth were modelled using a generalised 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 (cubic regression spline), conditioned by PFG order of arrival, 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, visualisation and analysis: R packages included in tidyverse (Wickham et al., 2019), car (Fox et al., 2019), Hmisc (Harrell, 2023), ggpubr (Kassambara, 2023), gtools (Bolker et al,. 2022), ggConvexHull (Martin, 2017) and viridis (Garnier et al., 2021).

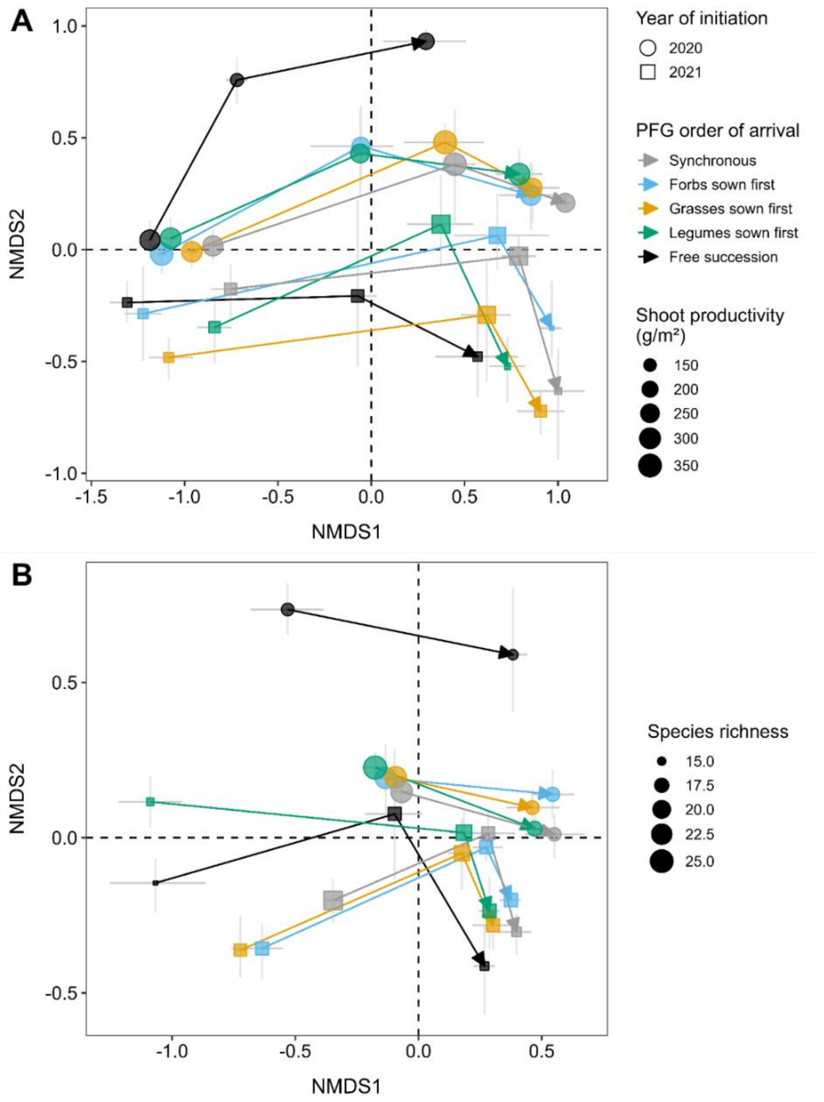


Figure 2. Time since establishment drives plant community composition more strongly than PFG order of arrival and year of initiation. Panels A and B are non-metric multidimensional scaling (NMDS) plots showing dissimilarities in plant community composition observed after one, two and three growing seasons 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 (gray segments) are shown (n=5).

Results

Time since establishment drives plant community composition more strongly than PFG order of arrival and year of initiation

Overall, time since establishment (partial R^2: 37-39%) had a much stronger effect on the composition of plant communities than PFG order of arrival (partial R^2: 8-10%) and the year of initiation of an experiment (partial R^2: 6-8%). This result was consistent for both biomass (Figure 2A, Table S3) and presence/absence data (Figure 2B, Table S4). The effect of PFG order of arrival on species composition was similar across combinations of year of initiation and sampling year (Figure 2A, $P = 0.082$; Figure 2B, $P=0.966$). 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 Tables S3-S4 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 year of initiation for community assembly in our POEM experiment (Figure 2, Tables S3-S4).

During the first growing season of both sub-experiments, plant communities were strongly dominated by unsown species, 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 (Figure S3). 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 (Figure S3). 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 (Figure S4). 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 (Figure S4).

Time since establishment, PFG order of arrival and year of initiation interactively modulate plant diversity

PFG order of arrival had distinct effects on plant diversity in each sub-experiment, particularly in the two first growing seasons (Figure 3, see Tables S5-7 for statistics). Plant species richness ($q=0$) was weakly affected by the year of initiation of an experiment, but was mainly dependent on PFG order of arrival and time since establishment (Figure 3, Table S5). At the end of the first growing season, we harvested on average seven species more in synchronous plots than in 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. When more weights are given to abundant species (i.e., at higher values of q), plant diversity decreased and did not differ between PFG arrival scenarios, except for forbs-first plots which were less diverse than synchronous plots at the end of the first growing season of POEM2021. When more weights are given to very dominant species ($q=2$), our plant communities were equivalent to an idealised assemblage with 3 (POEM2020) and 2 (POEM2021) equally abundant species, which is due to the strong dominance of the unsown species *Spergula arvensis* (58%), *Erodium*

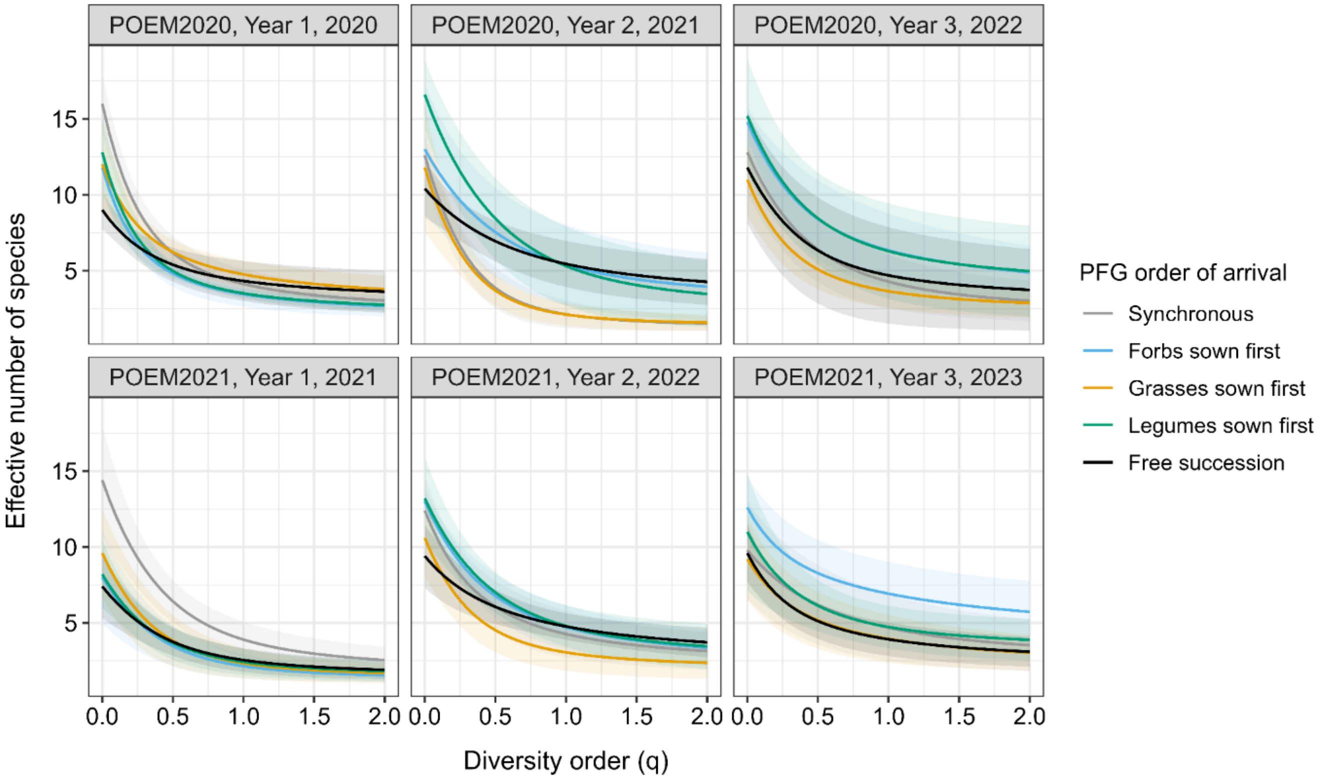


Figure 3. Time since establishment, PFG order of arrival and year of initiation interactively modulate plant diversity. These diversity profile plots show how the effective number of species (*D*) changes as a function of diversity order (*q*) for each combination of year of initiation, sampling year and PFG order of arrival. Lines and shaded areas show the average value and 95% confidence intervals (Gaussian) across five replicates, respectively. *D* values at q=0, q=1 and q=2 are equivalent to species richness, the exponential of Shannon entropy, and the inverse Simpson concentration, respectively. Increasing the value of *q* increases the weight of abundant species relative to rare ones. In terms of interpretation, if D=10 at q=0, this means that 10 species were present in the community (species richness). If D=3 at q=2, this means that the species diversity of order 2 of the community is equivalent to an idealised assemblage consisting of 3 equally abundant species (which would suggest that the assemblage contains 3 very dominant species). An assemblage whose species are all equally abundant (i.e., perfectly even assemblage) would be represented by a horizontal diversity profile (i.e., *D* equals to species richness for all values of *q*).

cicutarium (13%), *Anthemis arvensis* (12%) and *Chenopodium album* (6%) after one growing season (Figure 3).

After two growing seasons, we harvested on average four to seven species more in legumes-first plots than in free succession plots in both sub-experiments (Figure 3). Plant species richness, however, did not differ between plots where legumes were sown first and other PFG arrival scenarios, except for grasses-first plots in POEM2020 which had on average five species less than legumes-first plots. With greater weights for abundant (q=1) and dominant (q=2) species, plant diversity decreased more strongly in synchronous and grasses-first plots in

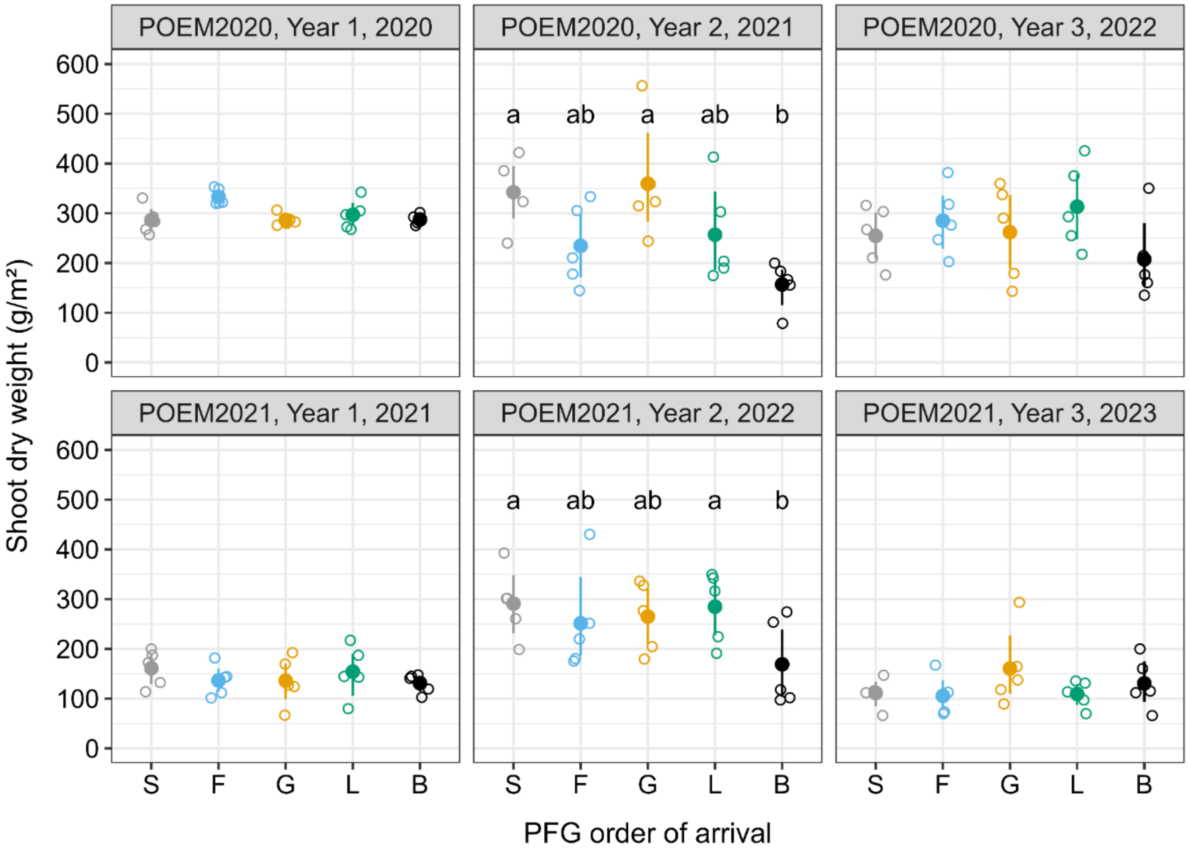


Figure 4. Year of initiation, not PFG order of arrival, is the main driver of aboveground productivity. For each combination of year of initiation, PFG of arrival and sampling year, the mean value (closed dot) and 95% confidence interval computed using non-parametric bootstrap are shown (n=5). Open dots 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.

POEM2020, which highlights 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, although grasses-first plots tended to be on average less diverse than the others (Figure 3).

Interestingly, towards the end of the third growing season, plots in which forbs were sown first became the most diverse (on average, four additional species collected at harvest) and had two times more common (q=1) and dominant species (q=2) than grasses-first plots (Figure 3). This result was observed in both sub-experiments. In POEM2020, we also found that legumes-first plots were more diverse than grasses-first plots. In POEM2021, plots in which forb species were sown first also contained two times more common and dominant species than free succession plots (Figure 3).

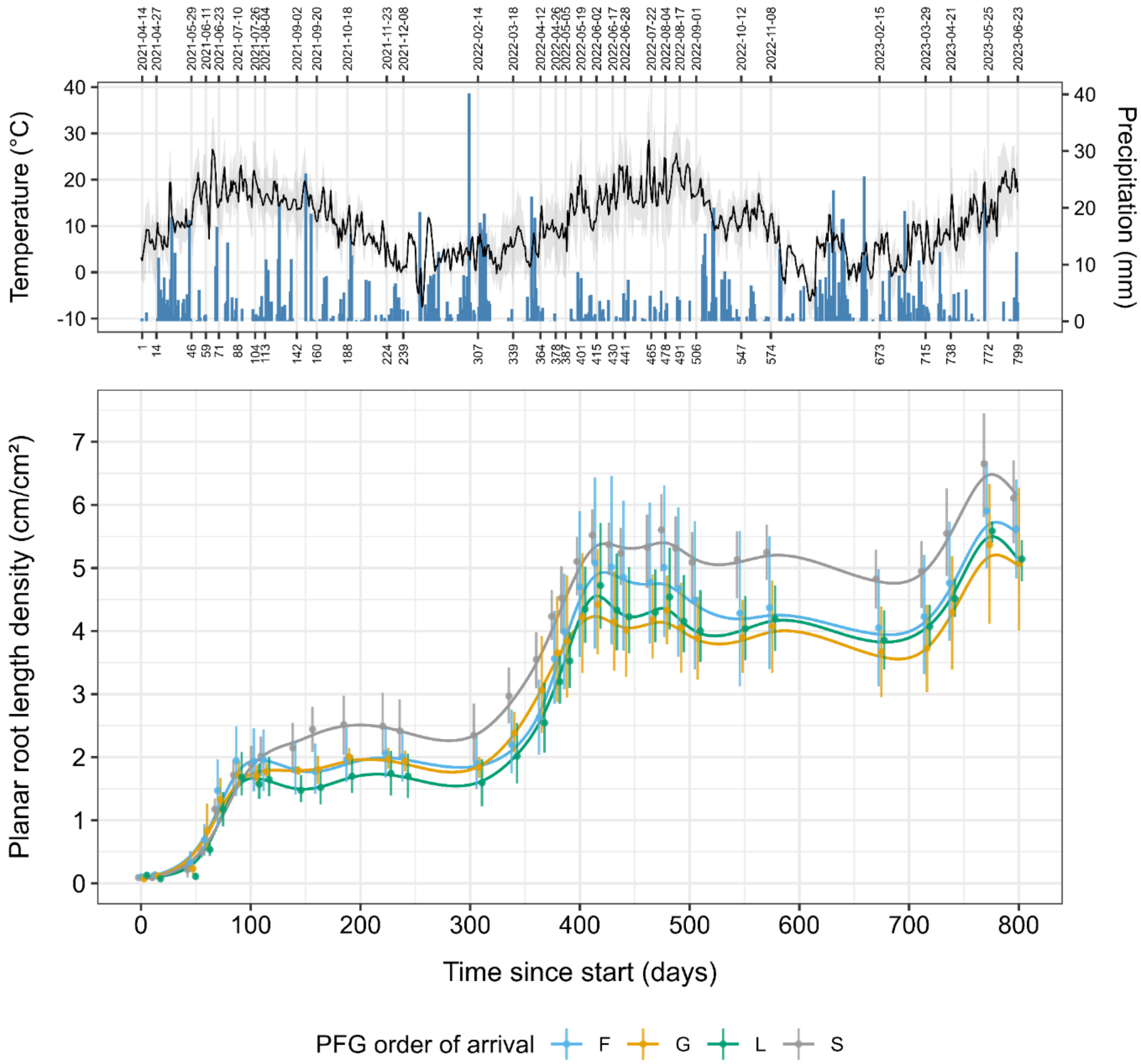


Figure 5. Root productivity was weakly affected by PFG order of arrival. Panel A shows the evolution of maximum, mean and minimum air temperature (°C), as well as daily precipitation (mm), at our experimental site between April 2021 and June 2023. Panel B shows the temporal evolution of the average planar root length density (*pRLD*) measured in POEM2021 plots for each PFG order of arrival scenario using minirhizotrons. Points and error bars indicate mean *pRLD* values and 95% confidence intervals (non-parametric bootstrap) measured at 33 time points spread over the first 800 days of POEM2021, respectively. Continuous lines are predictions from a generalised additive model. S, synchronous sowing of forbs, grasses and legumes; F, forbs sown first; G, grasses sown first; L, legumes sown first.

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

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 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 Table S8 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, Table S8).

Root productivity was weakly affected by PFG order of arrival

Using the average planar root length density (*pRLD*, Figure 5) and average planar root surface density (*pRSD*, Figure S5) measured in a plot using minirhizotron images as proxies for standing root biomass production, we found that PFG order of arrival did not 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

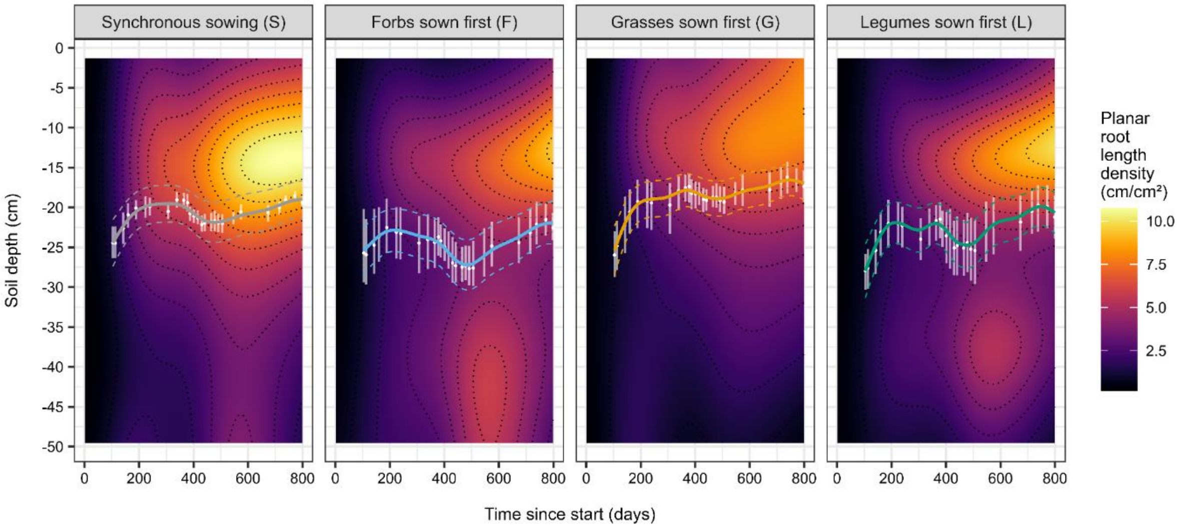


Figure 6. Sowing forbs or legumes first led to deeper-rooted plant communities. The raster image in each panel shows predictions from a first generalised additive model of the planar root length density (*pRLD*) as a function of time since the start of the experiment (0-800 days) and soil depth (1.4-49.5 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 generalised additive model.

synchronous plots was on average 13%, 24%, and 19% greater than in plots where forbs, grasses, or legumes were sown first.

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 (Figure 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 (Figure S6; 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 than in forbs-first, grasses-first or legumes-first communities (Figure 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 (Figures 6 and S6). 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).

Discussion

The POEM experiment enabled us to quantify the relative importance of PFG order of arrival, year of initiation and time since establishment for the structure and functioning of grassland plant communities during the first three years of community assembly. 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.

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 was dependent on time since establishment, but not year of initiation. 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 & 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 four years, (Stuble et al., 2017.b) 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 certainly be explained by the fact that, unlike (Stuble et al., 2017.b), 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 outcompeted the species sown in the plots. From the second growing season onwards, however, the sown species dominated the plots. This effect was probably influenced strongly 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 (Kirmer et al., 2018). Our findings are thus 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 (Bakker et al., 2019; Case et al., 2020; Chen et al., 2020; Herben et al., 2018; Lepik et al., 2021). In both POEM sub-experiments, the grasses-first treatment generally exhibited lower taxonomic diversity over the three growing seasons, possibly due to stronger competitive ability of grass species when given an initial advantage (Bakker et al., 2019, 2021; Chen et al., 2020), allowing them to outcompete other species and create stronger priority effects (Cadotte, 2023; Werner et al., 2016).

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 (Atkinson et al., 2023; J. D. Bakker et al., 2003; Catano et al., 2023; Stuble et al., 2017.a; Werner et al., 2020), 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, and (2) a too short sowing interval for nutrient-poor grasslands. For this reason, the next POEM sub-experiments will use a one year sowing interval between early- and late-arriving species groups.

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 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, e.g. in our study, root productivity was measured non-destructively over three years up to a depth of ~50 cm.

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. We found identical results when we manipulated PFG order of arrival 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 (Bakker et al., 2019; Case et al., 2020; Chen et al., 2020; Herben et al., 2018; 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., 2021, 2019; Chen et al., 2020), sowing grass species first may have increased root colonisation 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 sub-experiment with minirhizotron measurements will allow us to see whether and how our root distribution findings are affected by year of initiation.

Conclusions and outlook

In our study, 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 year of initiation or time since establishment had a stronger effect on aboveground community structure (composition and diversity) and functioning (aboveground biomass production). This is one of the few studies that experimentally looked at priority and year effects on both aboveground and belowground dynamics and our findings have potentially important implications for grassland restoration. If our findings can be generalised for a range of different grassland types, then sowing legumes or forbs-first could create communities with deeper roots that are more species-rich. This in turn could lead to plant communities that are more resistant/resilient to extreme weather events (e.g. drought) and potentially could improve soil carbon storage at depth. In order for our findings to be useful for grassland restoration, we need to better understand the mechanisms that determine the effects of plant order of arrival on root distribution, as well as identify the key environmental variables that drive the year effects that we found.

Acknowledgments

This work was funded by the German Research Foundation (project number: 420444099). The authors would like to thank Christoph Stegen for his exceptional technical support. Many thanks to all the colleagues and student helpers who helped us set up and maintain the POEM experiment.

Author Contributions

VMT, BMD and MS conceived the project, designed the experiment and secured funding; IMAC, BMD, TN and VMT collected data; IMAC and BMD analysed root images; IMAC, BMD and AF analysed data; IMAC produced the first draft of the manuscript, with support from BMD, AF and VMT. All authors contributed critically to the drafts and gave final approval for publication.

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.

References

Alonso-Crespo, I. M., Weidlich, E. W. A., Temperton, V. M., & Delory, B. M. (2023). Assembly history modulates vertical root distribution in a grassland experiment. *Oikos*. https://doi.org/10.1111/oik.08886

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Anderson, M. J., & Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, *83*(4), 557–574. https://doi.org/10.1890/12-2010.1

Atkinson, J., Groves, A. M., Towers, I. R., Catano, C. P., & Brudvig, L. A. (2023). Trait-mediated community assembly during experimental grassland restoration is altered by planting year rainfall. *The Journal of Applied Ecology*, *60*(8), 1587-1596. https://doi.org/10.1111/1365-2664.14430

Bakker, J. D., Wilson, S. D., Christian, J. M., Li, X., Ambrose, L. G., & Waddington, J. (2003). Contingency of grassland restoration on year, site, and competition from introduced grasses. *Ecological Applications: A Publication of the Ecological Society of America*, *13*(1), 137–153. https://doi.org/10.1890/1051-0761(2003)013[0137:cogroy]2.0.co;2

Bakker, L. M., Barry, K. E., Mommer, L., & van Ruijven, J. (2021). Focusing on individual plants to understand community scale biodiversity effects: the case of root distribution in grasslands. *Oikos* , *130*(11), 1954–1966. https://doi.org/10.1111/oik.08113

Bakker, L. M., Mommer, L., & van Ruijven, J. (2019). Using root traits to understand temporal changes in biodiversity effects in grassland mixtures. *Oikos* , *128*(2), 208–220. https://doi.org/10.1111/OIK.05612

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bolker B, Warnes G, Lumley T. (2022). *gtools: Various R Programming Tools. R package version 3.9.4,*. Comprehensive R Archive Network (CRAN). https://cran.r-project.org/web/packages/gtools/index.html

Cadotte, M. W. (2023). The interacting influences of competition, composition and diversity determine successional community change. *The Journal of Ecology*. https://doi.org/10.1111/1365-2745.14135

Case, M. F., Nippert, J. B., Holdo, R. M., & Staver, A. C. (2020). Root-niche separation between savanna trees and grasses is greater on sandier soils. *The Journal of Ecology*, *108*(6), 2298–2308. https://doi.org/10.1111/1365-2745.13475

Catano, C. P., Groves, A. M., & Brudvig, L. A. (2023). Community assembly history alters relationships between biodiversity and ecosystem functions during restoration. *Ecology*, *104*(2), e3910. https://doi.org/10.1002/ecy.3910

Chao, A., Chiu, C.-H., & Jost, L. (2014). Unifying Species Diversity, Phylogenetic Diversity, Functional Diversity, and Related Similarity and Differentiation Measures Through Hill Numbers. *Annual Review of Ecology, Evolution, and Systematics*, *45*(1), 297–324. https://doi.org/10.1146/annurev-ecolsys-120213-091540

Chase, J. M. (2003). Community assembly: when should history matter? *Oecologia*, *136*(4), 489–498. https://doi.org/10.1007/s00442-003-1311-7

Chen, B. J. W., Xu, C., Liu, M. S., Huang, Z. Y. X., Zhang, M. J., Tang, J., & Anten, N. P. R. (2020). Neighbourhood-dependent root distributions and the consequences on root separation in arid ecosystems. *The Journal of Ecology*, *108*(4), 1635–1648. https://doi.org/10.1111/1365-2745.13360

Dass, P., Houlton, B. Z., Wang, Y., & Warlind,

D. (2018). Grasslands may be more reliable carbon sinks than forests in California. *Environmental Research Letters: ERL [Web Site]*, *13*(7), 074027. https://doi.org/10.1088/1748-9326/aacb39

Delory, B. M., Weidlich, E. W. A., von Gillhaussen, P., & Temperton, V. M. (2019). When history matters: The overlooked role of priority effects in grassland overyielding. *Functional Ecology*, *33*(12), 2369–2380. https://doi.org/10.1111/1365-2435.13455

Drake, J. A. (1991). Community-Assembly Mechanics and the Structure of an Experimental Species Ensemble. *The American Naturalist*, *137*(1), 1–26. https://doi.org/10.1086/285143

Fox J, Weisberg S. (2019). *An R Companion to Applied Regression*. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Fukami, T. (2015). Historical contingency in community assembly : integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics*, *46*, 1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

Funk, J. L., Cleland, E. E., Suding, K. N., & Zavaleta, E. S. (2008). Restoration through reassembly: plant traits and invasion resistance. *Trends in Ecology & Evolution*, *23*(12), 695–703. https://doi.org/10.1016/j.tree.2008.07.013

Garnier, S., Ross, N., Rudis, R., Camargo, A. P., & Sciaini, M. (2021). Rvision-colorblind-friendly color maps for R. *R Package Version 0.6*.

Groves, A. M., Bauer, J. T., & Brudvig, L. A. (2020). Lasting signature of planting year weather on restored grasslands. *Scientific Reports*, *10*(1). https://doi.org/10.1038/S41598-020-62123-7

Groves, A. M., & Brudvig, L. A. (2019). Interannual variation in precipitation and other planting conditions impacts seedling establishment in sown plant communities. *Restoration Ecology*, *27*(1), 128–137. https://doi.org/10.1111/REC.12708

Harrell, F. E., Jr. (2023). *Harrell Miscellaneous [R package Hmisc version 5.1-1]*. https://CRAN.R-project.org/package=Hmisc

Herben, T., Vozábová, T., Hadincová, V., Krahulec, F., Mayerová, H., Pecháčková, S., Skálová, H., & Krak, K. (2018). Vertical root distribution of individual species in a mountain grassland community: Does it respond to neighbours? *The Journal of Ecology*, *106*(3), 1083–1095. https://doi.org/10.1111/1365-2745.12830

Hess, M. C. M., Mesléard, F., & Buisson, E. (2019). Priority effects: Emerging principles for invasive plant species management. *Ecological Engineering*, *127*, 48–57. https://doi.org/10.1016/j.ecoleng.2018.11.011

Kassambara, A. (2023, February 10). *ggpubr: "ggplot2" Based Publication Ready Plots. R package version 0.6.0*. Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=ggpubr

Kirmer, A., Rydgren, K., & Tischew, S. (2018). Smart management is key for successful diversification of field margins in highly productive farmland. *Agriculture, Ecosystems & Environment*, *251*, 88–98. https://doi.org/10.1016/j.agee.2017.09.028

Körner, C., Stöcklin, J., Reuther-Thiébaud, L., & Pelaez-Riedl, S. (2008). Small differences in arrival time influence composition and productivity of plant communities. *The New Phytologist*, *177*(3), 698–705. https://doi.org/10.1111/j.1469-8137.2007.02287.x

Lambers, J. H. R., Harpole, W. S., Tilman, D., Knops, J., & Reich, P. B. (2004). Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. *Ecology Letters*, *7*(8), 661–668. https://doi.org/10.1111/j.1461-0248.2004.00623.x

Lenth, R. V. (2023). *Estimated Marginal Means, aka Least-Squares Means [R package emmeans version 1.8.8]*. https://CRAN.R-project.org/package=emmeans

Lepik, A., Abakumova, M., Davison, J., Zobel, K., & Semchenko, M. (2021). Spatial mapping of root systems reveals diverse strategies of soil exploration and resource contest in grassland plants. *The Journal of Ecology*, *109*(2), 652–663. https://doi.org/10.1111/1365-2745.13535

Li, D. (2018). hillR: taxonomic, functional, and phylogenetic diversity and similarity through Hill Numbers. *Journal of Open Source Software*, *3*(31), 1041. https://doi.org/10.21105/joss.01041

Linder, H. P., Lehmann, C. E. R., Archibald, S., Osborne, C. P., & Richardson, D. M. (2018). Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. *Biological Reviews of the Cambridge Philosophical Society*, *93*(2), 1125–1144. https://doi.org/10.1111/brv.12388

Lyons, K. G., Török, P., Hermann, J.-M., Kiehl, K., Kirmer, A., Kollmann, J., Overbeck, G. E., Tischew, S., Allen, E. B., Bakker, J. D., Brigham, C., Buisson, E., Crawford, K., Dunwiddie, P., Firn, J., Grobert, D., Hickman, K., Stradic, S. L. E., & Temperton, V. M. (2023). Challenges and opportunities for grassland restoration: A global perspective of best practices in the era of climate change. *Global Ecology and Conservation*, *46*, e02612. https://doi.org/10.1016/j.gecco.2023.e02612

MacDougall, A. S., Wilson, S. D., & Bakker, J. D. (2008). Climatic variability alters the outcome of long-term community assembly. *The Journal of Ecology*, *96*(2), 346–354. https://doi.org/10.1111/j.1365-2745.2007.01333.x

Martin, C. (2017). *ggConvexHull: Add a convex hull geom to ggplot2*. Github. https://github.com/cmartin/ggConvexHull

Martin, L. M., & Wilsey, B. J. (2012). Assembly history alters alpha and beta diversity, exotic-native proportions and functioning of restored prairie plant communities. *The Journal of Applied Ecology*, *49*(6), 1436–1445. https://doi.org/10.1111/j.1365-2664.2012.02202.x

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J. (2022). *Community Ecology Package [R package vegan version 2.6-4]*. Comprehensive R Archive Network (CRAN). https://cran.r-project.org/web/packages/vegan/index.html

R Core Team. (2023). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.r-project.org/*. R Foundation for Statistical Computing. https://www.R-project.org/

Seethepalli, A., Dhakal, K., Griffiths, M., Guo, H., Freschet, G. T., & York, L. M. (2021). RhizoVision Explorer: open-source software for root image analysis and measurement standardization. *AoB Plants*, *13*(6), lab056. https://doi.org/10.1093/aobpla/plab056

Smith, A. G., Han, E., Petersen, J., Olsen, N. A. F., Giese, C., Athmann, M., Dresbøll, D. B., & Thorup-Kristensen, K. (2022). RootPainter: deep learning segmentation of biological images with corrective annotation. *The New Phytologist*, *236*(2), 774–791. https://doi.org/10.1111/nph.18387

Staude, I. R., Segar, J., Temperton, V. M., Andrade, B. O., de Sá Dechoum, M., Weidlich, E. W. A., & Overbeck, G. E. (2023). Prioritize grassland restoration to bend the curve of biodiversity loss. *Restoration Ecology*, *31*(5). https://doi.org/10.1111/rec.13931

Stuble, K. L., Fick, S. E., & Young, T. P. (2017.a). Every restoration is unique: testing year effects and site effects as drivers of initial restoration trajectories. *The Journal of Applied Ecology*, *54*(4), 1051–1057. https://doi.org/10.1111/1365-2664.12861

Stuble, K. L., Zefferman, E. P., Wolf, K. M., Vaughn, K. J., & Young, T. P. (2017.b). Outside the envelope: rare events disrupt the relationshipbetween climate factors and species interactions. *Ecology*, *98*(6), 1623–1630. https://doi.org/10.1002/ecy.1820

Temperton, V. M., Buchmann, N., Buisson, E., Durigan, G., Kazmierczak, L., Perring, M. P., Sá Dechoum, M., Veldman, J. W., & Overbeck, G. E. (2019). Step back from the forest and step up to the Bonn Challenge: How a broad ecological perspective can promote successful landscape restoration. *Restoration Ecology*, *rec.12989*. https://doi.org/10.1111/rec.12989

von Gillhaussen, P., Rascher, U., Jablonowski, N. D., Plückers, C., Beierkuhnlein, C., & Temperton, V. M. (2014). Priority effects of time of arrival of plant functional groups override sowing interval or density effects: a grassland experiment. *PloS One*, *9*(1), e86906. https://doi.org/10.1371/journal.pone.0086906

Wagemaker, C. A. M., Mommer, L., Visser, E. J. W., Weigelt, A., van Gurp, T. P., Postuma, M., Smit-Tiekstra, A. E., & de Kroon, H. (2020). msGBS: A new high-throughput approach to quantify the relative species abundance in root samples of multispecies plant communities. *Molecular Ecology Resources*, *21*(4), 1021–1036. https://doi.org/10.1111/1755-0998.13278

Warton D.I, Wright ST, & Wang Y. (2012). *Distance-based multivariate analyses confound location and dispersion effects*. https://doi.org/10.1111/j.2041-210X.2011.00127.x

Weidlich, E. W. A., Nelson, C. R., Maron, J. L., Callaway, R. M., Delory, B. M., & Temperton, V. M. (2021). Priority effects and ecological restoration. *Restoration Ecology*, *29*(1), e13317. https://doi.org/10.1111/REC.13317

Weidlich, E. W. A., von Gillhaussen, P., Delory, B. M., Blossfeld, S., Poorter, H., & Temperton, V. M. (2017). The Importance of Being First: Exploring Priority and Diversity Effects in a Grassland Field Experiment. *Frontiers in Plant Science*, *7*(2008), 1–12. https://doi.org/10.3389/fpls.2016.02008

Weidlich, E. W. A., von Gillhaussen, P., Max, J. F. J., Delory, B. M., Jablonowski, N. D., Rascher, U., & Temperton, V. M. (2018). Priority effects caused by plant order of arrival affect belowground productivity. *The Journal of Ecology*, *106*, 774–780. https://doi.org/10.1111/1365-2745.12829

Weisser, W. W., Roscher, C., Meyer, S. T., Ebeling, A., Luo, G., Allan, E., Beßler, H., Barnard, R. L., Buchmann, N., Buscot, F., Engels, C., Fischer, C., Fischer, M., Gessler, A., Gleixner, G., Halle, S., Hildebrandt, A., Hillebrand, H., de Kroon, H., … Eisenhauer, N. (2017). Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic and Applied Ecology*, *23*, 1–73. https://doi.org/10.1016/j.baae.2017.06.002

Werner, C. M., Stuble, K. L., Groves, A. M., & Young, T. P. (2020). Year effects: Interannual variation as a driver of community assembly dynamics. *Ecology*, *101*(9), e03104. https://doi.org/10.1002/ecy.3104

Werner, C. M., Vaughn, K. J., Stuble, K. L., Wolf, K., & Young, T. P. (2016). Persistent asymmetrical priority effects in a California grassland restoration experiment. *Ecological Applications: A*

Publication of the Ecological Society of America, *26*(6), 1624–1632. https://doi.org/10.1890/15-1918.1

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wilsey, B. (2020). Restoration in the face of changing climate: importance of persistence, priority effects, and species diversity. *Restoration Ecology*, *rec.13132*. https://doi.org/10.1111/rec.13132

Wood, S. (2017). mgcv-package Mixed GAM Computation Vehicle with GCV/AIC/REML smoothness estimation and GAMMs by REML/PQL. *Docs.W3cub.Com*. https://docs.w3cub.com/r/library/mgcv/html/mgcv-package

Yannelli, F. A., MacLaren, C., & Kollmann, J. (2020). Moving Away From Limiting Similarity During Restoration: Timing of Arrival and Native Biomass Are Better Proxies of Invasion Suppression in Grassland Communities. *Frontiers in Ecology and Evolution*, *8*. https://doi.org/10.3389/fevo.2020.00238

2.1 Supplementary information of Chapter 1

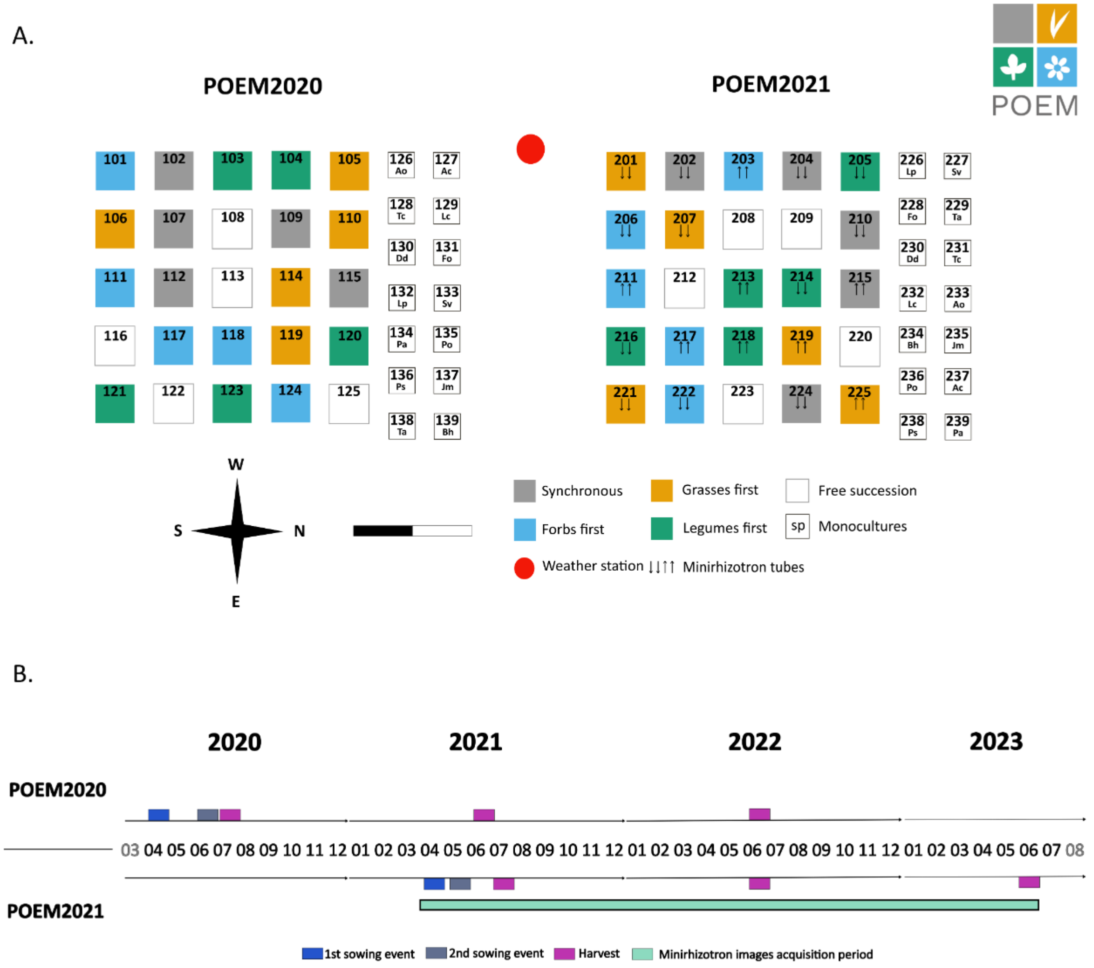


Figure S1. Overview of the POEM experiment. A. Experimental design. As of June 2023, the experiment consists of two sub-experiments set up in different years: POEM2020 (set up in 2020) and POEM2021 (set up in 2021). Each sub-experiment consists of 25 mixture plots (3×3 m²) and 14 monoculture plots (3×3 m²). 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*. **B. Experimental timeline**. This timeline includes activities carried out in POEM2020 and POEM2021 during the first three growing seasons.

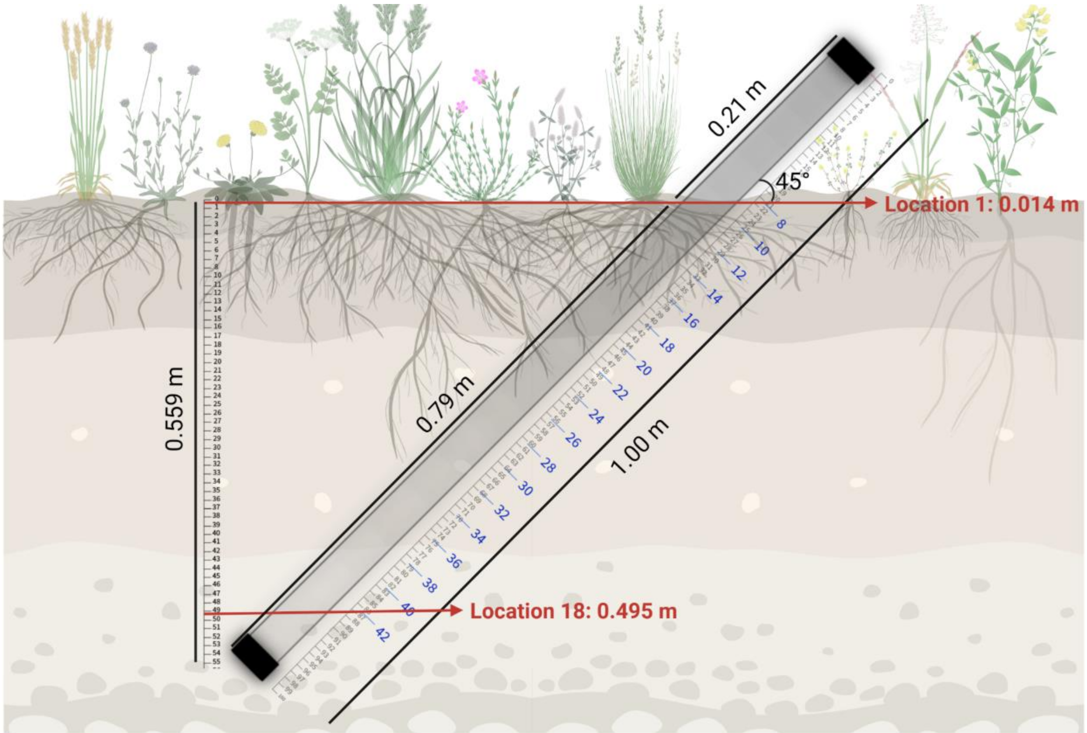


Figure S2. Installation of minirhizotron tubes in POEM2021 plots. Minirhizotron tubes were installed at a 45° angle. A step-by-step description of the installation of minirhizotron tubes in POEM2021 is available in the video provided as supplementary material. Roots growing along a minirhizotron tube were regularly imaged at 18 equally spaced locations (in blue) along the tube. Using the setup shown in the figure, root images were collected between 1.4 cm depth and 49.5 cm depth.

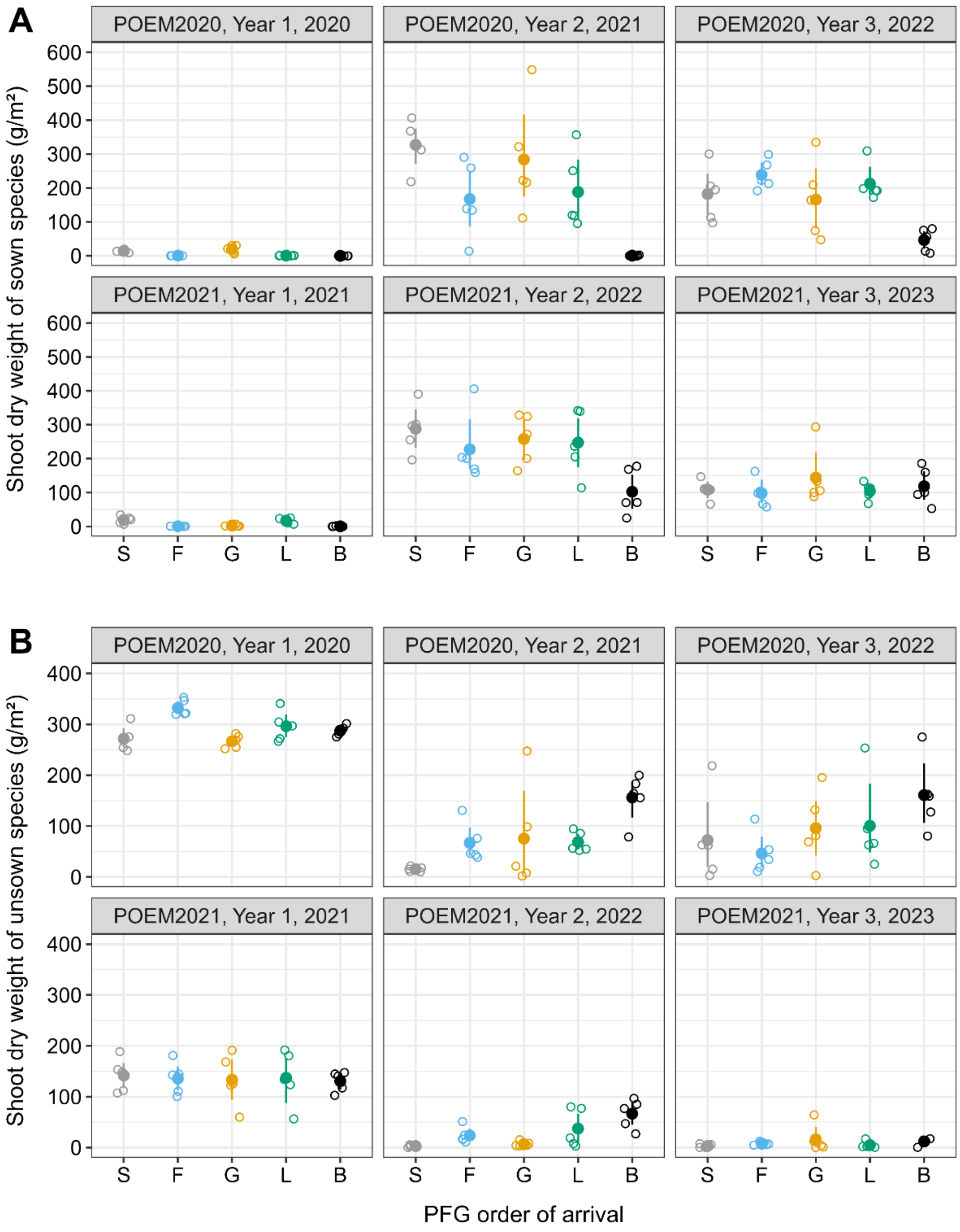


Figure S3. Effects of year of initiation, sampling year and PFG order of arrival on the aboveground productivity of sown (A) and unsown (B) species. For each combination of year of initiation, PFG of arrival and sampling year, the mean value (closed dot) and 95% confidence interval computed using non-parametric 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.

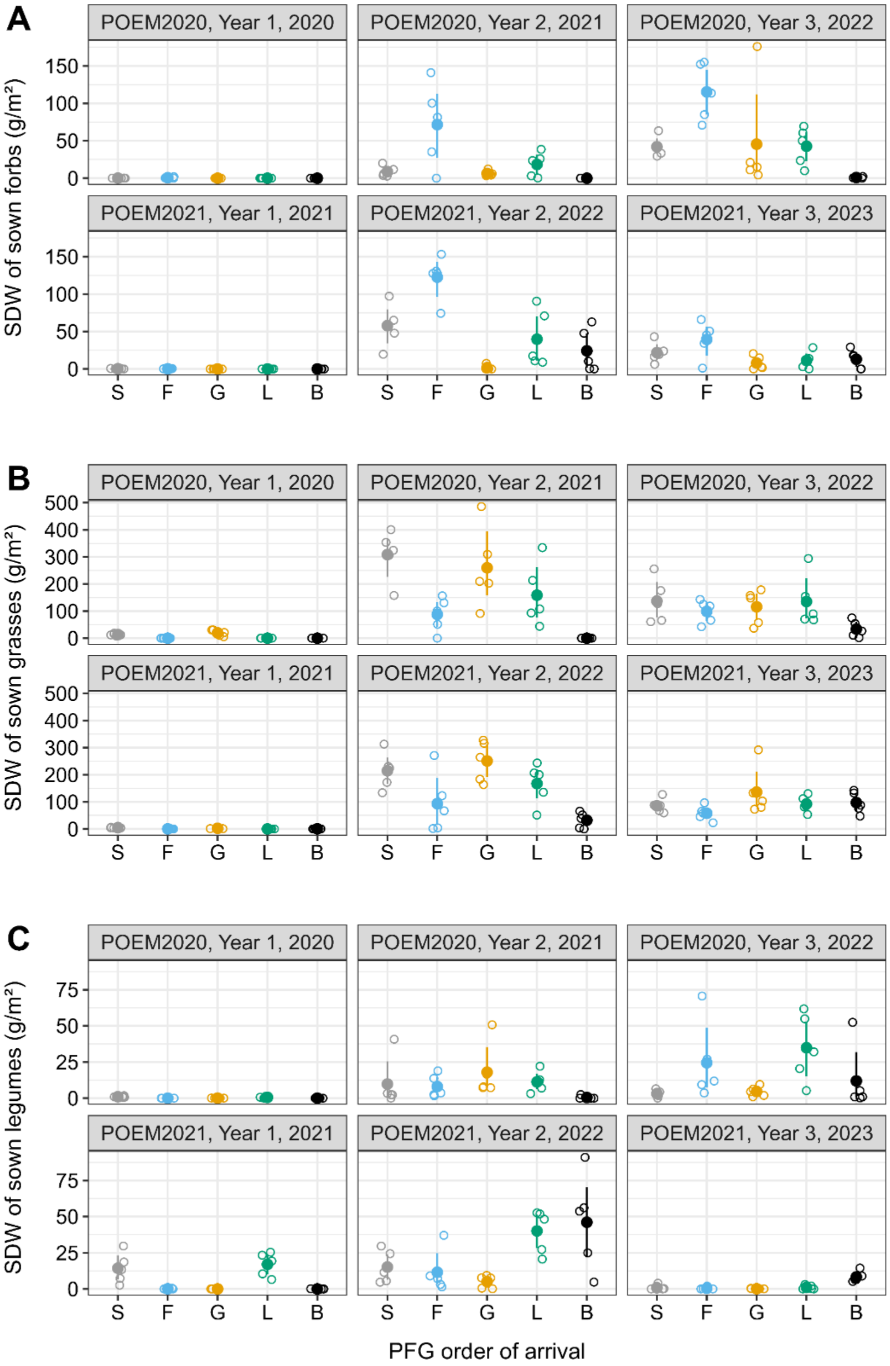


Figure S4. Effects of year of initiation, sampling year and PFG order of arrival on the aboveground productivity of sown forbs (A), grasses (B) and legumes (C). For each combination of year of initiation, PFG of arrival and sampling year, the mean value (closed dot) and 95% confidence interval computed 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.

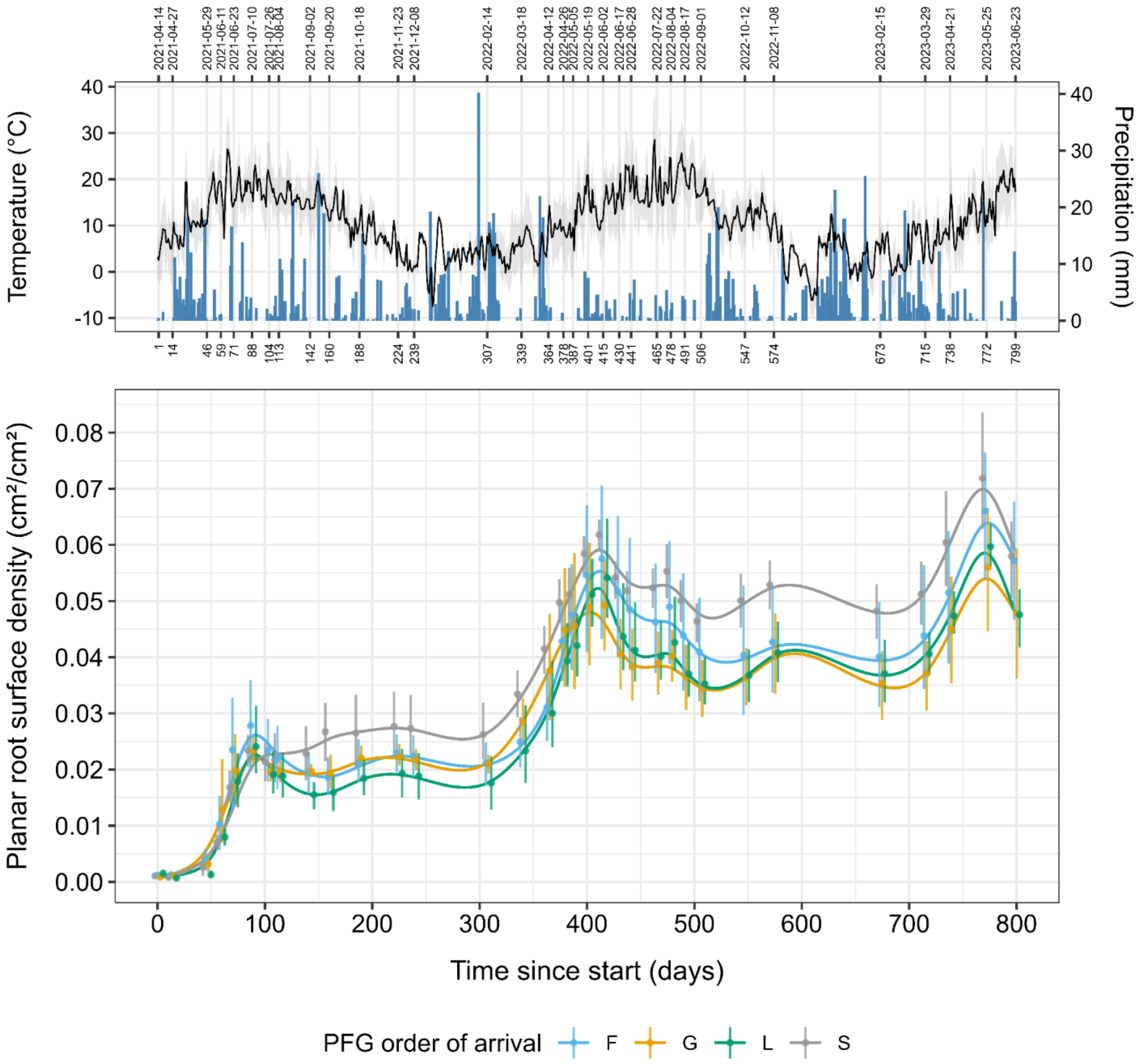


Figure S5. Root productivity was weakly affected by PFG order of arrival. Panel A shows the evolution of maximum, mean and minimum air temperature (°C), as well as daily precipitation (mm), at our experimental site between April 2021 and June 2023. Panel B shows the temporal evolution of the average planar root surface density (*pRSD*) measured in POEM2021 plots for each PFG order of arrival scenario using minirhizotrons. Points and error bars indicate mean *pRSD* values and 95% confidence intervals (non-parametric bootstrap) measured at 33 time points spread over the first 800 days of POEM2021, respectively. Continuous lines are predictions from a generalised additive model. S, synchronous sowing of forbs, grasses and legumes; F, forbs sown first; G, grasses sown first; L, legumes sown first.

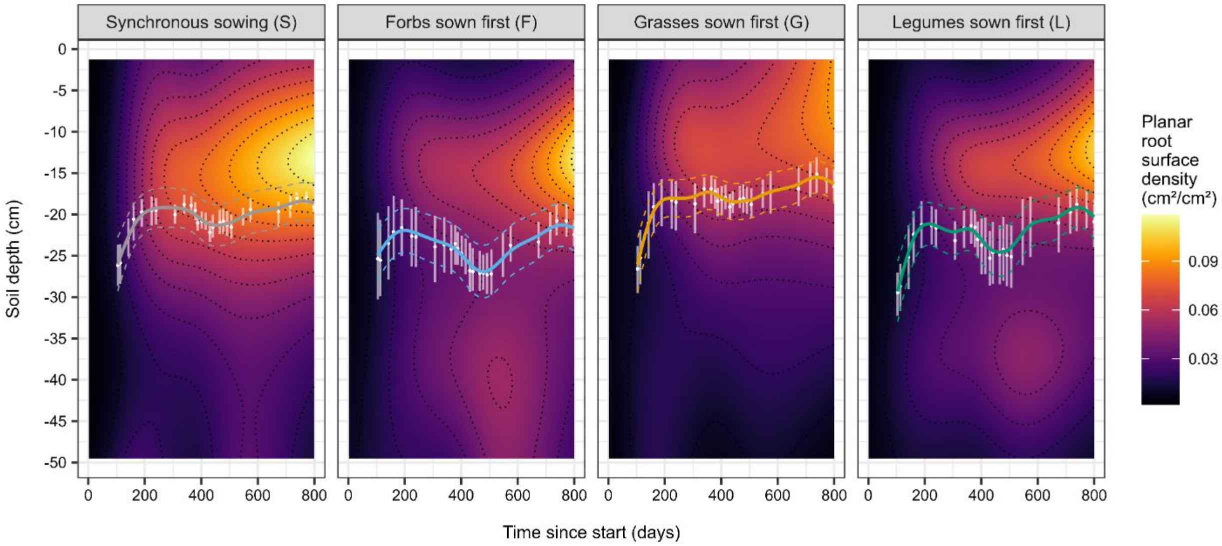


Figure S6. Sowing forbs or legumes first led to deeper-rooted plant communities. The raster image in each panel shows predictions from a first generalised additive model of the planar root surface density (*pRSD*) as a function of time since the start of the experiment (0-800 days) and soil depth (1.4-49.5 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 surface density data between days 100 and 800, respectively. Continuous lines are predicted *MRD* values from a second generalised additive model.

Table S1. Composition of the seed mixtures used in the mixture plots of POEM2020 and POEM2021. For each species, the target number of individuals in a plot was calculated considering (1) the plot surface area (9 m²), (2) the desired plant density (1000 plants/m²), (3) the fact that PFGs should be equally represented in the seed mixture, and (4) the fact that species within each PFG should be equally represented in the seed mixture. 'Seeds per plot' is the total number of seeds needed to reach the target number of individuals of a species in a plot. 'Seed mass per plot' is the corresponding seed mass value. TSW, thousand seed weight (g); FG, functional group (F for forbs, G for grasses, L for legumes); GR, germination rate [0,1].

Species	**FG**	**Target number of ind per plot**	**POEM2020**				**POEM2021**			
			TSW (g)	GR	Seeds per plot	Seed mass per plot (g)	TSW (g)	GR	Seeds per plot	Seed mass per plot (g)
Lotus corniculatus	L	750	1.25	0.71	1050	1.31	1.36	0.76	987	1.34
Trifolium arvense	L	750	0.31	0.31	2386	0.74	0.43	0.51	1463	0.63
Trifolium campestre	L	750	0.49	0.31	2386	1.18	0.53	0.56	1339	0.71
Lathyrus pratensis	L	750	10.65	0.51	1458	15.54	9.40	0.68	1099	10.33
Anthoxanthum odoratum	G	750	0.51	0.40	1875	0.95	0.47	0.48	1579	0.74
Bromus hordeaceus	G	750	2.55	0.74	1010	2.57	2.70	0.52	1442	3.89
Festuca ovina	G	750	0.28	0.31	2386	0.67	0.25	0.16	4688	1.18
Agrostis capillaris	G	750	0.07	0.86	875	0.06	0.09	0.28	2727	0.25
Silene vulgaris	F	500	0.59	0.77	648	0.38	0.55	0.88	568	0.31
Dianthus deltoides	F	500	0.16	0.89	565	0.09	0.18	0.92	543	0.10
Pilosella officinarum	F	500	0.19	0.76	658	0.12	0.19	0.92	543	0.10
Jasione montana	F	500	0.02	0.74	673	0.01	0.02	0.50	1000	0.02
Potentilla argentea	F	500	0.13	0.51	972	0.12	0.07	0.24	2083	0.16
Pimpinella saxifraga	F	500	0.73	0.13	3846	2.81	0.93	0.20	2500	2.32

Table S2. Seed mass used in the monoculture plots of POEM2020 and POEM2021. The target number of individuals in a plot was calculated considering (1) the plot surface area (4 m²) and (2) the desired plant density (1000 plants/m²). ‘Seeds per plot’ is the total number of seeds needed to reach the target number of individuals of a species in a plot. ‘Seed mass per plot’ is the corresponding seed mass value. TSW, thousand seed weight (g); FG, functional group (F for forbs, G for grasses, L for legumes); GR, germination rate [0,1].

Species	**FG**	**Target number of ind per plot**	**POEM2020**				**POEM2021**			
			TSW (g)	GR	Seeds per plot	Seed mass per plot (g)	TSW (g)	GR	Seeds per plot	Seed mass per plot (g)
Lotus corniculatus	L	4000	1.25	0.71	5600	6.97	1.36	0.76	5263	7.16
Trifolium arvense	L	4000	0.31	0.31	12727	3.95	0.43	0.51	7805	3.35
Trifolium campestre	L	4000	0.49	0.31	12727	6.27	0.53	0.56	7143	3.78
Lathyrus pratensis	L	4000	10.65	0.51	7778	82.87	9.40	0.68	5861	55.09
Anthoxanthum odoratum	G	4000	0.51	0.40	10000	5.08	0.47	0.48	8421	3.97
Bromus hordeaceus	G	4000	2.55	0.74	5385	13.72	2.70	0.52	7692	20.75
Festuca ovina	G	4000	0.28	0.31	12727	3.58	0.25	0.16	25000	6.29
Agrostis capillaris	G	4000	0.07	0.86	4667	0.33	0.09	0.28	14545	1.34
Silene vulgaris	F	4000	0.59	0.77	5185	3.07	0.55	0.88	4545	2.48
Dianthus deltoides	F	4000	0.16	0.89	4516	0.72	0.18	0.92	4348	0.77
Pilosella officinarum	F	4000	0.19	0.76	5263	0.98	0.19	0.92	4348	0.81
Jasione montana	F	4000	0.02	0.74	5385	0.10	0.02	0.50	8000	0.18
Potentilla argentea	F	4000	0.13	0.51	7778	0.97	0.07	0.24	16667	1.24
Pimpinella saxifraga	F	4000	0.73	0.13	30769	22.46	0.93	0.20	20000	18.60

Table S3. Results of permutational multivariate analysis of variance (PERMANOVA) testing the effects of PFG order of arrival, year of initiation and sampling year on dissimilarities in plant species composition using species-specific biomass data. The Bray-Curtis dissimilarity index was used. Terms were added sequentially (first to last). Number of permutations: 1000. See also figure 2A.

	Df	Sum of squares	R^2	F	P-value
PFG order of arrival (a)	4	3.46	**0.077**	7.94	**0.000999**
Sampling year (b)	2	17.70	**0.393**	81.22	**0.000999**
Year of initiation (c)	1	2.61	**0.058**	23.97	**0.000999**
a*b	8	2.79	**0.062**	3.20	**0.000999**
a*c	4	1.09	**0.024**	2.50	**0.002997**
b*c	2	3.11	**0.069**	14.27	**0.000999**
a*b*c	8	1.15	0.026	1.32	0.081918
Residual	120	13.07	0.291		
Total	149	44.98	1.000		

Table S4. Results of permutational multivariate analysis of variance (PERMANOVA) testing the effects of PFG order of arrival, year of initiation and sampling year on dissimilarities in plant species composition using presence/absence data. The Jaccard dissimilarity index was used. Terms were added sequentially (first to last). Number of permutations: 1000. See also figure 2B.

	Df	Sum of squares	R^2	F	P-value
PFG order of arrival (a)	4	1.75	**0.097**	8.23	**0.000999**
Sampling year (b)	2	6.76	**0.373**	63.50	**0.000999**
Year of initiation (c)	1	1.48	**0.081**	27.72	**0.000999**
a*b	8	1.03	**0.057**	2.42	**0.000999**
a*c	4	0.52	**0.029**	2.47	**0.000999**
b*c	1	1.12	**0.062**	21.03	**0.000999**
a*b*c	4	0.12	0.007	0.59	0.966034
Residual	100	5.32	0.294		
Total	124	18.11	1.000		

Table S5. Results of a generalised linear mixed-effect model testing the effects of PFG order of arrival, year of initiation and sampling year on the effective number of species at *q*=0 (species richness). Plot ID was used as a random effect in the model (variance: 0.0049). The model was fitted using a Gamma distribution and a log-link function. This analysis of deviance table (Type III Wald chi square tests) was produced using the Anova function in the car package (Fox and Weisberg, 2019). See also Figure 3.

	Chisq	Df	P-value
(Intercept)	1027.20	1	**< 2.2e-16**
PFG order of arrival (a)	22.53	4	**0.0002**
Sampling year (b)	6.91	2	**0.0316**
Year of initiation (c)	0.96	1	0.3262
a*b	24.52	8	**0.0019**
a*c	5.03	4	0.2843
b*c	3.41	2	0.1817
a*b*c	6.01	8	0.6461

Table S6. Results of a generalised linear mixed-effect model testing the effects of PFG order of arrival, year of initiation and sampling year on the effective number of species at *q*=1. Plot ID was used as a random effect in the model (variance: 0.0127). The model was fitted using a Gamma distribution and a log-link function. This analysis of deviance table (Type III Wald chi square tests) was produced using the Anova function in the car package (Fox and Weisberg, 2019). See also Figure 3.

	Chisq	Df	P-value
(Intercept)	111.93	1	**< 2.2e-16**
PFG order of arrival (a)	3.93	4	0.4149
Sampling year (b)	27.15	2	**1.27e-06**
Year of initiation (c)	0.11	1	0.7370
a*b	73.79	8	**8.62e-13**
a*c	6.59	4	0.1591
b*c	14.21	2	**0.0008**
a*b*c	16.97	8	**0.0304**

Table S7. Results of a generalised linear mixed-effect model testing the effects of PFG order of arrival, year of initiation and sampling year on the effective number of species at *q*=2. Plot ID was used as a random effect in the model (variance: 0.0174). The model was fitted using a Gamma distribution and a log-link function. This analysis of deviance table (Type III Wald chi square tests) was produced using the Anova function in the car package (Fox and Weisberg, 2019). See also Figure 3.

	Chisq	Df	P-value
(Intercept)	56.18	1	**6.60e-14**
PFG order of arrival (a)	4.06	4	0.3979
Sampling year (b)	23.68	2	**7.21e-06**
Year of initiation (c)	0.83	1	0.3628
a*b	61.76	8	**2.10e-10**
a*c	4.81	4	0.3073
b*c	15.74	2	**0.0004**
a*b*c	15.33	8	0.0530

Table S8. Results of a generalised linear mixed-effect model testing the effects of PFG order of arrival, year of initiation and sampling year on total shoot dry weight (g/m²). Plot ID was used as a random effect in the model (variance: 0.0091). The model was fitted using a Gamma distribution and a log-link function. This analysis of deviance table (Type III Wald chi square tests) was produced using the Anova function in the car package (Fox and Weisberg, 2019). See also Figure 4.

	Chisq	Df	P-value
(Intercept)	1963.29	1	**< 2.2e-16**
PFG order of arrival (a)	1.01	4	0.9077
Sampling year (b)	3.58	2	0.1669
Year of initiation (c)	9.97	1	**0.0016**
a*b	24.85	8	**0.0016**
a*c	1.96	4	0.7433
b*c	9.04	2	**0.0109**
a*b*c	16.90	8	**0.0312**

2.2 Supplementary photographic material of Chapter 1

POEM2020

MARCH 2020
Poem field side before fence installation

APRIL 2020
POEM2020 plots

27th APRIL 2020
1st sowing of POEM2020 plots

8th JUNE 2020
2nd sowing of POEM2020 plots

MAY 2020
POEM2020 plots

3rd JUNE 2020
POEM2020 plots

25th JUNE 2020
POEM2020 plots

28th JUNE 2021
POEM2020 plots

MAY 2022
POEM2020 plots

JUNE 2023
POEM2020 plots

POEM2021

SEPTEMBER 2020
Secale cerale removal for POEM2021 plots installation

OCTOBER 2020
Plot marking and minirhizotron tubes installation

OCTOBER 2020
Minirhizotron tubes installed in POEM2021 plots

NOVEMBER 2020
POEM2021 cover to prevent species growing

APRIL 2021
POEM2021 ground sheet removal

APRIL 2021
Snowy POEM2021 plots

13th APRIL 2021
1st sowing of POEM2021 plots

25th MAY 2022
2nd sowing of POEM2021 plots

15th JUNE 2021
POEM2021 plots

MAY 2022
POEM2021 plots

JUNE 2023
POEM2021 plots

Chapter 2

Assembly history modulates vertical root distribution in a grassland experiment

Chapter 2

Assembly history modulates vertical root distribution in a grassland experiment

Published article: Oikos, 2023(1), e08886 https://doi.org/10.1111/oik.08886

Inés M. Alonso-Crespo, Emanuela W.A. Weidlich, Vicky M. Temperton and Benjamin M. Delory

Abstract

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 com- munities 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

OIKOS

Research article

Assembly history modulates vertical root distribution in a grassland experiment

Inés M. Alonso-Crespo[1], Emanuela W. A. Weidlich[1], Vicky M. Temperton[1] and Benjamin M. Delory[1]

[1]Inst. of Ecology, Leuphana Univ., Lüneburg, Germany

Correspondence: Benjamin M. Delory (benjamin.delory@leuphana.de)

Oikos
2023: e08886
doi: 10.1111/oik.08886
Subject Editor and
Editor-in-Chief:
Gerlinde B. De Deyn
Accepted 12 February 2022

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

NORDIC SOCIETY OIKOS

www.oikosjournal.org

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

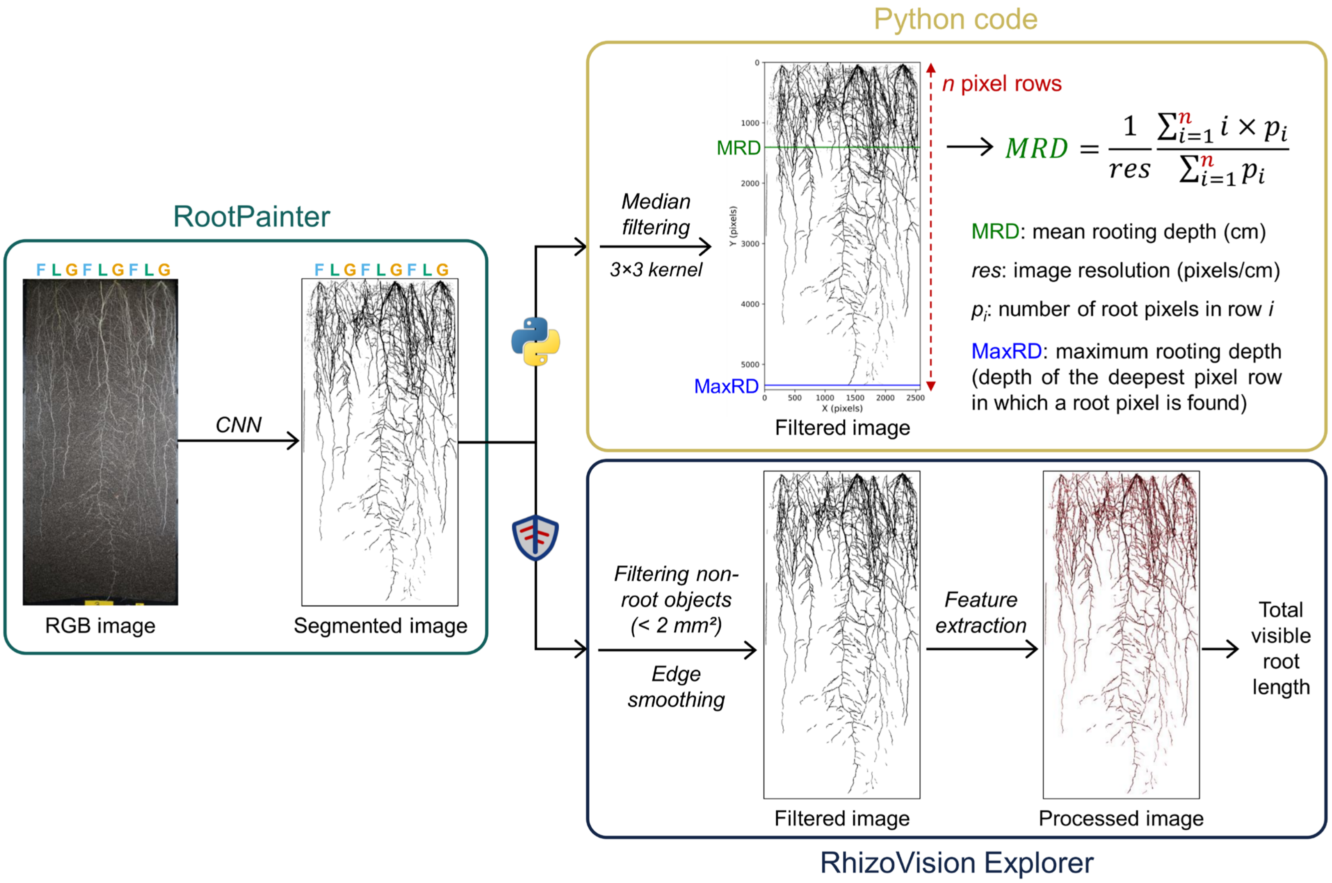


Figure 1. Description of the image analysis pipeline used for non-destructively estimating the total visible root length and the mean (MRD) and maximum (MaxRD) rooting depth of plant communities growing in rhizoboxes. Our approach included four main steps: 1) training a convolutional neural network (CNN) 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 ith pixel row multiplied by the depth of the ith 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

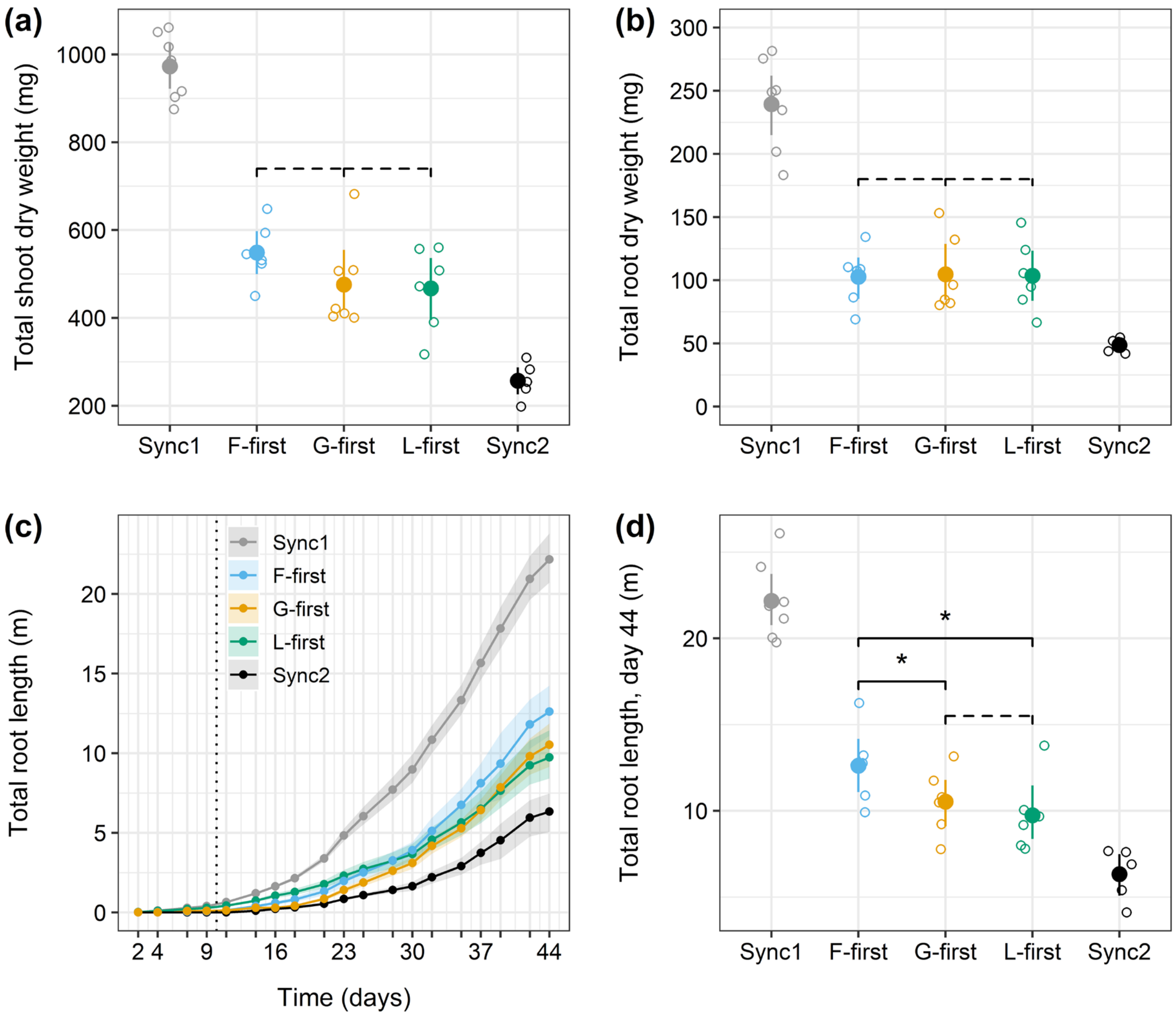


Figure 2. Effect of PFG order of arrival on the total shoot productivity (a), total root productivity (b) and total visible root length (c–d) of plant communities. For each treatment, mean values (solid dots) and compatibility intervals (solid bars in panels a, b and d; shaded areas in panel c) computed 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

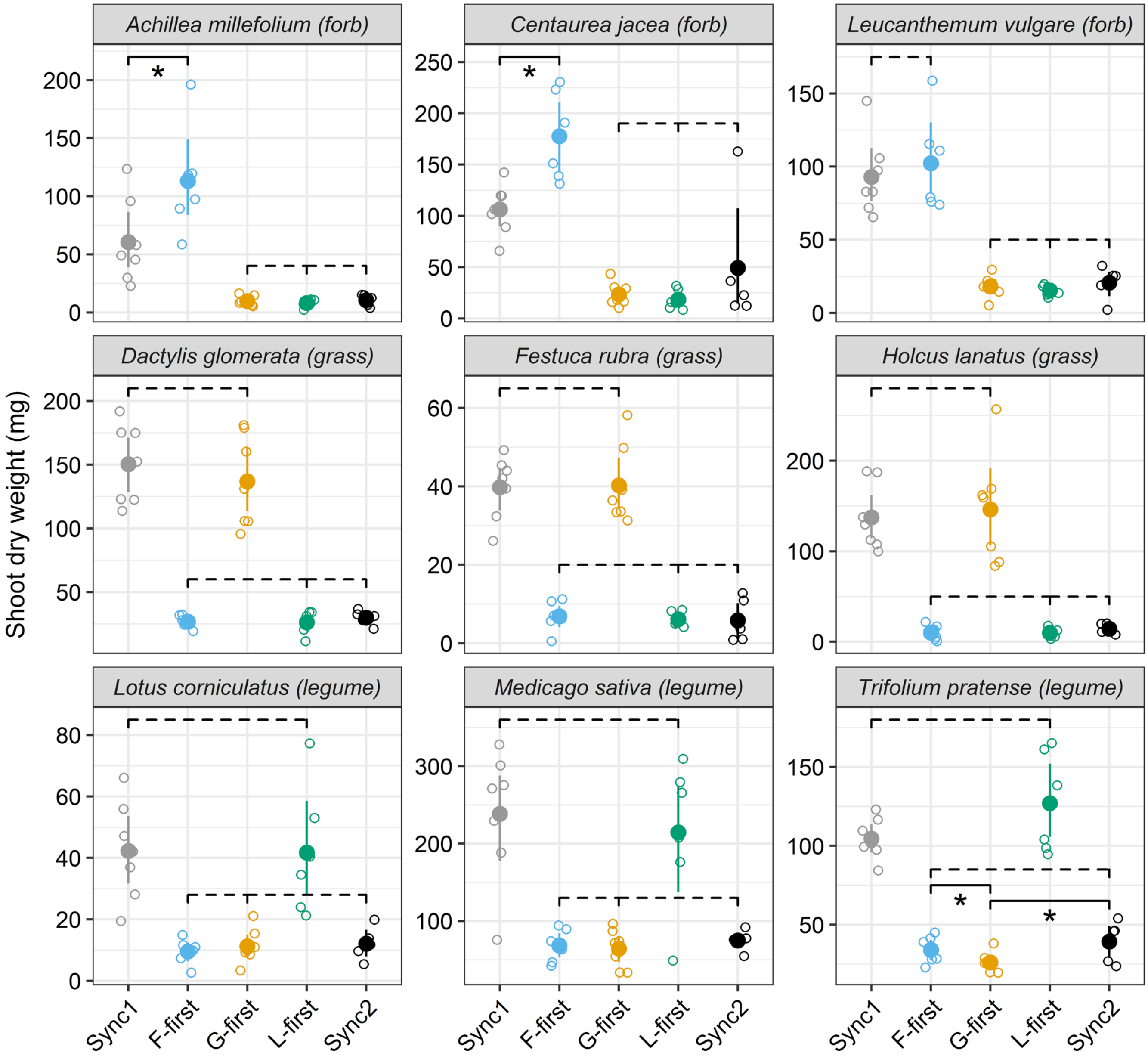


Figure 3. Effect of PFG order of arrival on the shoot productivity of individual species. For each treatment, mean values (solid dots) and compatibility intervals (solid bars) computed using non-parametric bootstrap are shown (n = 5–7). Individual species observations are displayed as open dots. In all 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

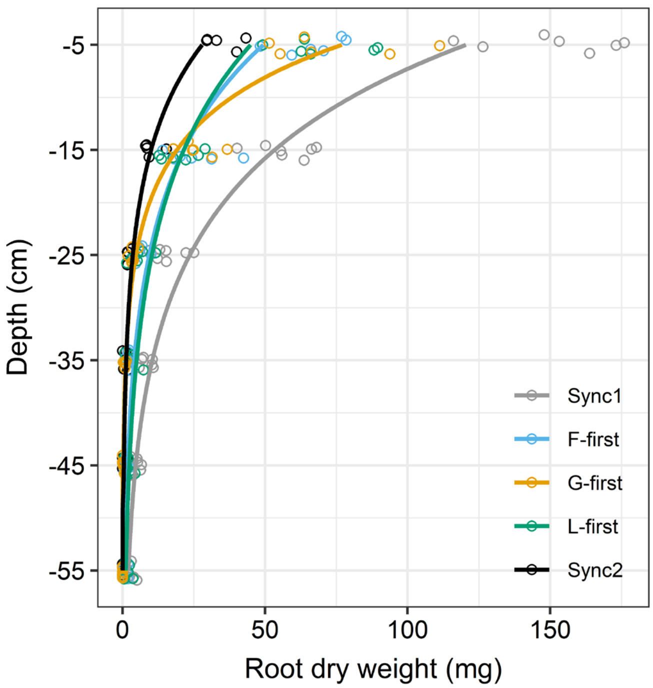


Figure 4. Vertical root biomass distribution in the soil is affected by PFG order of arrival. Lines represent ZAG model fits for each PFG order of arrival scenario (i.e. predictions obtained after merging the gamma and binomial components of the ZAG model). Predictions of the gamma and binomial components 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

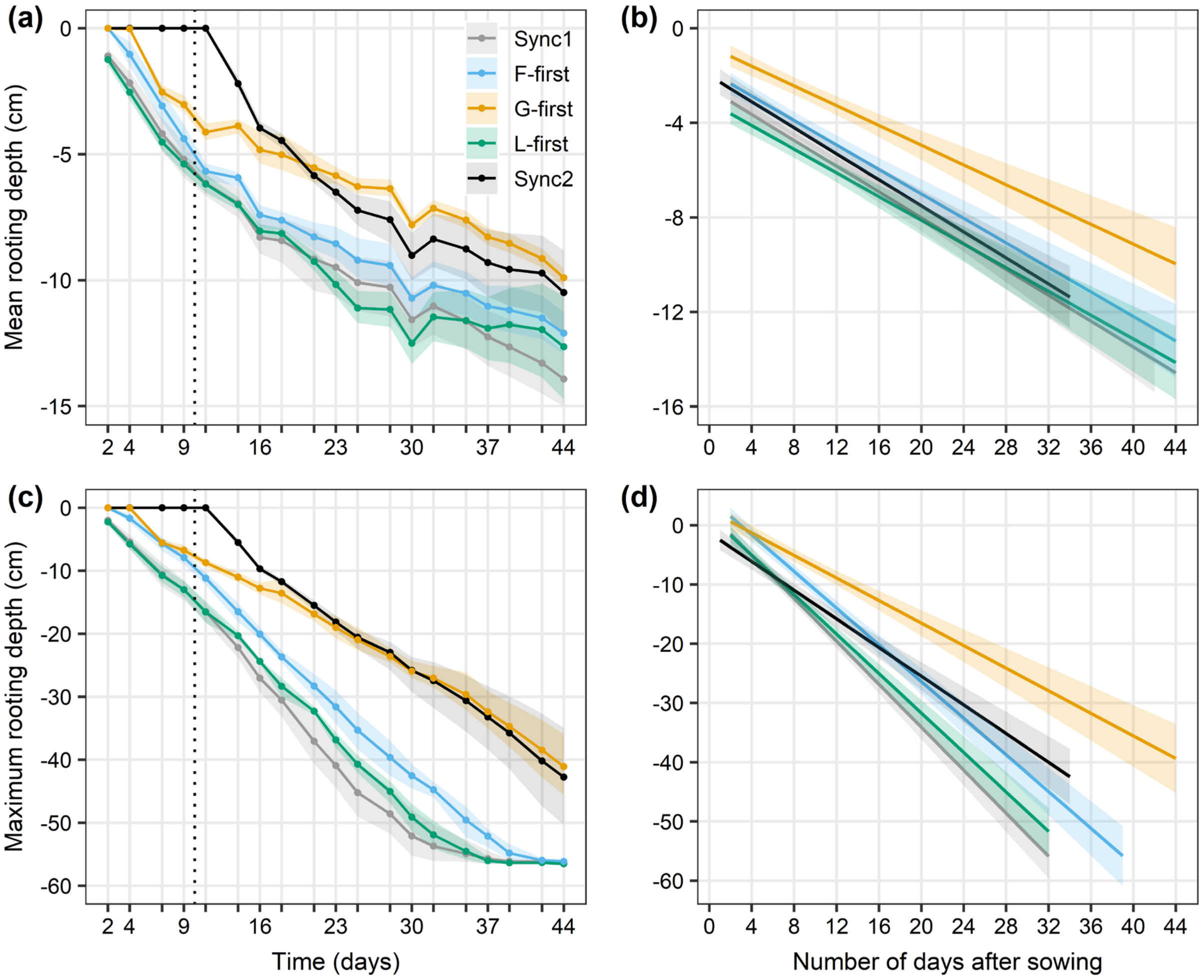


Figure 5. The temporal evolution of rooting depth is affected by PFG order of arrival. (a) Temporal evolution of the mean rooting depth (MRD) of plant communities for each PFG order of arrival scenario; (b) linear mixed-effect model fits describing the temporal evolution of MRD for each plant community 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.

Acknowledgements – The authors would like to thank Dr Thomas Niemeyer (Leuphana University of Lüneburg, Germany) for his outstanding technical support and Abraham George Smith (University of Copenhagen, Denmark) for his extensive advice that enabled us to successfully use RootPainter to analyse our root images. Open access funding enabled and organized by Projekt DEAL.
Funding – This research was funded by the Chair of Ecosystem Functioning and Services of the Leuphana University of Lüneburg (Germany). IMAC, VMT and BMD acknowledge funding from the German Research Foundation (project no.: 420444099 and 470604360).

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.

References

Ahlmann-Eltze, C. and Patil, I. 2021. ggsignif: R package for displaying significance brackets for 'ggplot2.' – PsyArxiv.
Alonso-Crespo, I. M. et al. 2021. Data from: Assembly history modulates vertical root distribution in a grassland experiment. – Zenodo Digital Repository, https://doi.org/10.5281/zenodo.5713397.
Amrhein, V. et al. 2019. Scientists rise up against statistical significance. – Nature 567: 305–307.
Bakker, L. M. et al. 2019. Using root traits to understand temporal changes in biodiversity effects in grassland mixtures. – Oikos 128: 208–220.
Bakker, L. M. et al. 2021. Focusing on individual plants to understand community scale biodiversity effects: the case of root distribution in grasslands. – Oikos 130: 1954–1966.
Barry, K. E. et al. 2020. Limited evidence for spatial resource partitioning across temperate grassland biodiversity experiments. – Ecology 101: e02905.
Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1–48.
Case, M. F. et al. 2020. Root-niche separation between savanna trees and grasses is greater on sandier soils. – J. Ecol. 108: 2298–2308.
Chase, J. M. 2003. Community assembly: when should history matter? – Oecologia 136: 489–498.
Chen, B. J. W. et al. 2020. Neighbourhood-dependent root distributions and the consequences on root separation in arid ecosystems. – J. Ecol. 108: 1635–1648.
de Kroon, H. et al. 2012. Root responses to nutrients and soil biota: drivers of species coexistence and ecosystem productivity. – J. Ecol. 100: 6–15.
Delory, B. M. et al. 2018. Measuring plant root traits under controlled and field conditions: step-by-step procedures. – In: Ristova, D. and Barbez, E. (eds), Root development: methods and protocols. Springer, pp. 3–22.
Delory, B. M. et al. 2019a. The exotic species *Senecio inaequidens* pays the price for arriving late in temperate European grassland communities. – Oecologia 191: 657–671.
Delory, B. M. et al. 2019b. When history matters: the overlooked role of priority effects in grassland overyielding. – Funct. Ecol. 33: 2369–2380.
Delory, B. M. et al. 2021. Soil chemical legacies trigger species-specific and context-dependent root responses in later arriving plants. – Plant Cell Environ. 44: 1215–1230.

Ejrnaes, R. et al. 2006. Community assembly in experimental grasslands: suitable environment or timely arrival? – Ecology 87: 1225–1233.

Freschet, G. T. et al. 2021. A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. – New Phytol. 232: 973–1122.

Fukami, T. 2015. Historical contingency in community assembly: integrating niches, species pools and priority effects. – Annu. Rev. Ecol. Evol. Syst. 46: 1–23.

Fukami, T. and Nakajima, M. 2011. Community assembly: alternative stable states or alternative transient states? – Ecol. Lett. 14: 973–984.

Fukami, T. et al. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. – Ecol. Lett. 13: 675–684.

Grman, E. and Suding, K. N. 2010. Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. – Restor. Ecol. 18: 664–670.

Herben, T. et al. 2018. Vertical root distribution of individual species in a mountain grassland community: does it respond to neighbours? – J. Ecol. 106: 1083–1095.

Hess, M. C. M. et al. 2019. Priority effects: emerging principles for invasive plant species management. – Ecol. Eng. 127: 48–57.

Hess, M. C. M. et al. 2020. Giving recipient communities a greater head start and including productive species boosts early resistance to invasion. – Appl. Veg. Sci. 23: 340–352.

Jesch, A. et al. 2018. Below-ground resource partitioning alone cannot explain the biodiversity–ecosystem function relationship: a field test using multiple tracers. – J. Ecol. 106: 2002–2018.

Kardol, P. et al. 2007. Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. – Ecol. Monogr. 77: 147–162.

Kardol, P. et al. 2013. Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. – Oikos 122: 84–94.

Kassambara, A. 2020. ggpubr: 'ggplot2' based publication ready plots. – R package ver. 0.4.0. https://CRAN.R-project.org/package=ggpubr.

Körner, C. et al. 2008. Small differences in arrival time influence composition and productivity of plant communities. – New Phytol. 177: 698–705.

Kraft, N. J. B. et al. 2015. Plant functional traits and the multidimensional nature of species coexistence. – Proc. Natl Acad. Sci. USA 112: 797–802.

Kumar, A. et al. 2020. Barley shoot biomass responds strongly to N:P stoichiometry and intraspecific competition, whereas roots only alter their foraging. – Plant Soil 453: 515–528.

Lepik, A. et al. 2021. Spatial mapping of root systems reveals diverse strategies of soil exploration and resource contest in grassland plants. – J. Ecol. 109: 652–663.

Lüdecke, D. 2018. ggeffects: tidy data frames of marginal effects from regression models. – J. Open Source Softw. 3: 772.

Luo, W. et al. 2021. Limited evidence of vertical fine-root segregation in a subtropical forest. – New Phytol. 231: 2308–2318.

Mahall, B. E. and Callaway, R. M. 1991. Root communication among desert shrubs. – Proc. Natl Acad. Sci. USA 88: 874–876.

Martin, L. M. and Wilsey, B. J. 2012. Assembly history alters alpha and beta diversity, exotic-native proportions and functioning of restored prairie plant communities. – J. Appl. Ecol. 49: 1436–1445.

Mommer, L. et al. 2010. Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. – J. Ecol. 98: 1117–1127.

Mommer, L. et al. 2012. Interactive effects of nutrient heterogeneity and competition: implications for root foraging theory? – Funct. Ecol. 26: 66–73.

Mueller, K. E. et al. 2013. Root depth distribution and the diversity–productivity relationship in a long-term grassland experiment. – Ecology 94: 787–793.

Muff, S. et al. 2021. Rewriting results sections in the language of evidence. – Trends Ecol. Evol. 37: 203–210.

Oram, N. J. et al. 2018. Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. – J. Ecol. 106: 265–277.

Peng, S. and Chen, H. Y. H. 2021. Global responses of fine root biomass and traits to plant species mixtures in terrestrial ecosystems. – Global Ecol. Biogeogr. 30: 289–304.

Poorter, H. et al. 2015. How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. – New Phytol. 208: 736–749.

Ravenek, J. M. et al. 2014. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. – Oikos 123: 1528–1536.

Ravenek, J. M. et al. 2016. Linking root traits and competitive success in grassland species. – Plant Soil 407: 39–53.

Rewald, B. and Ephrath, J. E. 2013. Minirhizotron techniques. – In: Eshel, A. and Beeckman, T. (eds), Plant roots: the hidden half. CRC Press, pp. 42.1–42.16.

Rillig, M. C. et al. 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. – Science 366: 886–890.

Sarneel, J. M. et al. 2016. The importance of priority effects for riparian plant community dynamics. – J. Veg. Sci. 27: 658–667.

Schindelin, J. et al. 2012. Fiji – an open source platform for biological image analysis. – Nat. Methods 9: 676–682.

Seethepalli, A. et al. 2021. RhizoVision Explorer: open-source software for root image analysis and measurement standardization. – AoB Plants 13: lab056.

Semchenko, M. et al. 2007. Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. – New Phytol. 176: 644–654.

Smith, A. G. et al. 2020. RootPainter: deep learning segmentation of biological images with corrective annotation. – bioRxiv: 2020.04.16.044461.

Steiner, C. F. and Leibold, M. A. 2004. Cyclic assembly trajectories and scale-dependent productivity–diversity relationships. – Ecology 85: 107–113.

Sutherland, J. P. 1974. Multiple stable points in natural communities. – Am. Nat. 108: 859–873.

Temperton, V. M. et al. 2016. Assembly theory for restoring ecosystem structure and functioning: timing is everything? – In: Palmer, M. A. et al. (eds), Foundations of restoration ecology, 2nd edn. Island Press, pp. 245–270.

Valverde-Barrantes, O. J. et al. 2015. Aggregated and complementary: symmetric proliferation, overyielding and mass effects explain fine-root biomass in soil patches in a diverse temperate deciduous forest landscape. – New Phytol. 205: 731–742.

Vannette, R. L. and Fukami, T. 2014. Historical contingency in species interactions: towards niche-based predictions. – Ecol. Lett. 17: 115–124.

Venables, W. N. and Ripley, B. D. 2002. Modern applied statistics with S. – Springer.

von Gillhaussen, P. et al. 2014. Priority effects of time of arrival of plant functional groups override sowing interval or density effects: a grassland experiment. – PLoS One 9: e86906.

Wagemaker, C. A. M. et al. 2021. msGBS: a new high-throughput approach to quantify the relative species abundance in root samples of multispecies plant communities. – Mol. Ecol. Resour. 21: 1021–1036.

Wasserstein, R. L. et al. 2019. Moving to a world beyond 'p < 0.05.' – Am. Stat. 73: 1–19.

Weidlich, E. W. A. et al. 2017. The importance of being first: exploring priority and diversity effects in a grassland field experiment. – Front. Plant Sci. 7: 2008.

Weidlich, E. W. A. et al. 2018a. Priority effects caused by plant order of arrival affect below-ground productivity. – J. Ecol. 106: 774–780.

Weidlich, E. W. A. et al. 2018b. Neighbourhood stories: role of neighbour identity, spatial location and order of arrival in legume and non-legume initial interactions. – Plant Soil 424: 171–182.

Weidlich, E. W. A. et al. 2021. Priority effects and ecological restoration. – Restor. Ecol. 29: e13317.

Weigelt, A. et al. 2021. An integrated framework of plant form and function: the belowground perspective. – New Phytol. 232: 42–59.

Werner, C. M. et al. 2016. Persistent asymmetrical priority effects in a California grassland restoration experiment. – Ecol. Appl. 26: 1624–1632.

Werner, G. D. A. and Kiers, E. T. 2015. Order of arrival structures arbuscular mycorrhizal colonization of plants. – New Phytol. 205: 1515–1524.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. – Springer.

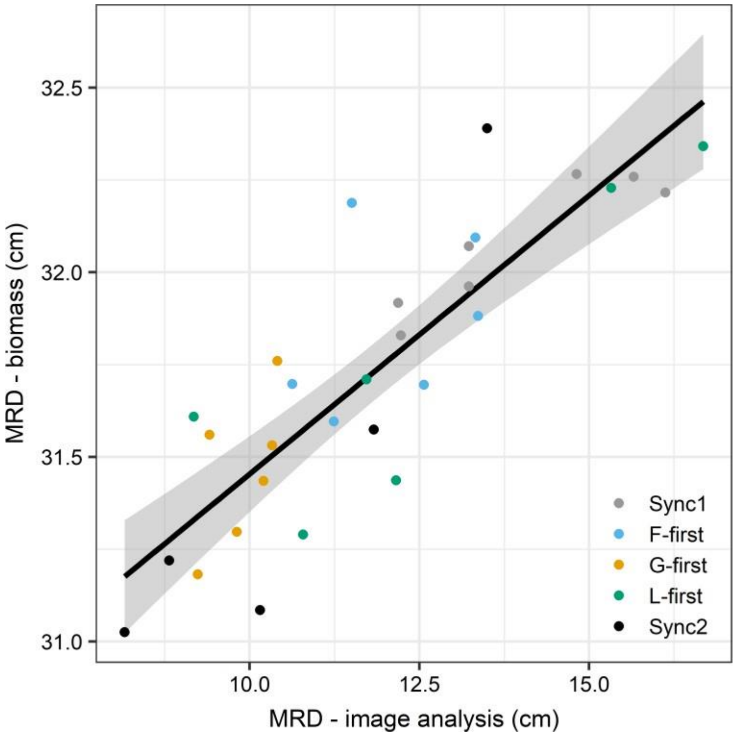
Yannelli, F. A. et al. 2020. Moving away from limiting similarity during restoration: timing of arrival and native biomass are better proxies of invasion suppression in grassland communities. – Front. Ecol. Evol. 8: 238.

Young, T. P. et al. 2017. Using priority effects to manipulate competitive relationships in restoration. – Restor. Ecol. 25: S114–S123.

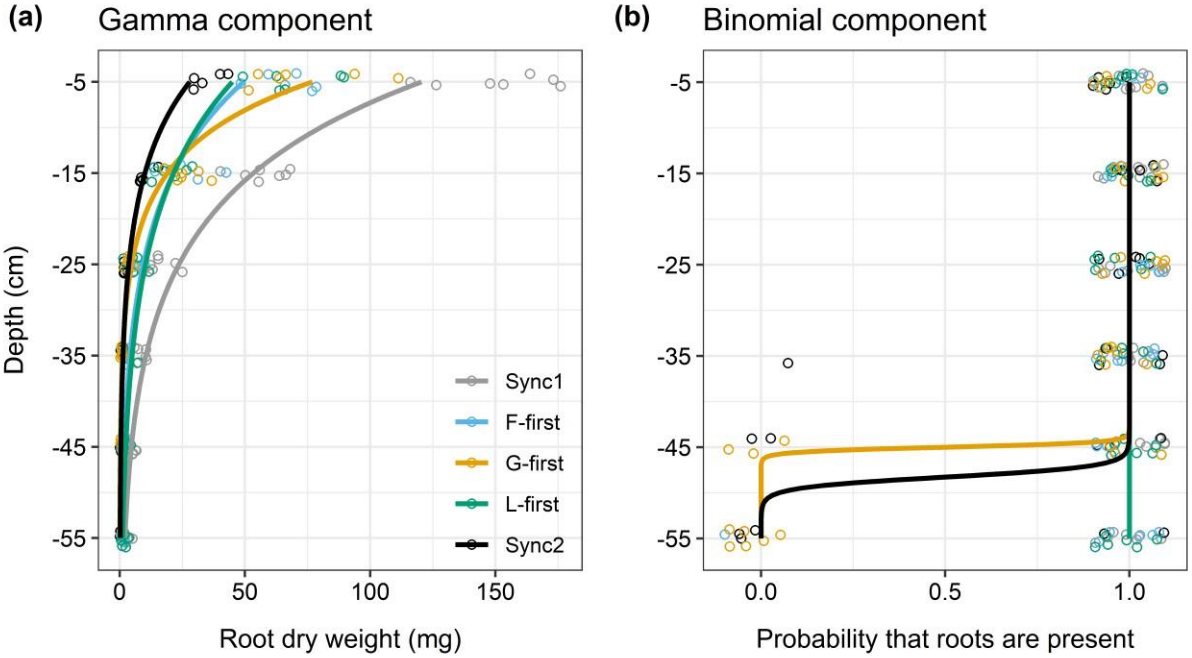
Zeng, W. et al. 2021. Positive tree diversity effect on fine root biomass: via density dependence rather than spatial root partitioning. – Oikos 130: 1–14.

Zuur, A. F. and Ieno, E. N. 2016. Beginner's guide to zero-inflated models with R. – Highland Statistics Ltd.

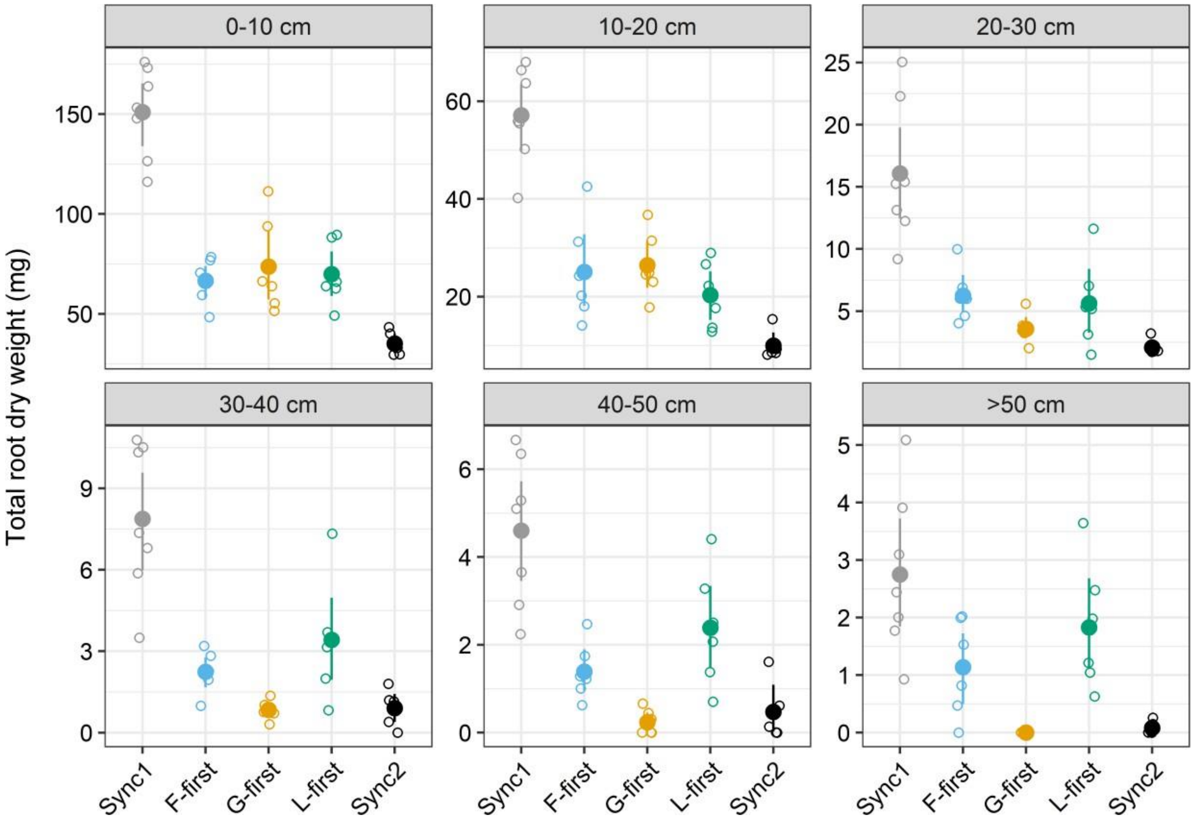
3.1 Supplementary information of Chapter 2



Supplementary Fig. 1. Relationship between mean rooting depth (MRD) values computed using root biomass and MRD values computed using image analysis (r=0.86, P < 0.001) at harvest (day 44). Individual observations are displayed as solid dots (n=5-7). 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.



Supplementary Fig. 2. Predictions of the gamma (a) and binomial (b) components ofthe zero-altered gamma model (ZAG model, also referred to as hurdle model) describing how plant functional group order of arrival affected the vertical distribution of the root biomass in our rhizoboxes (*n*=5-7). ZAG predictions (i.e., predictions obtained after merging the gamma and binomial components of the model) are shown in Figure 4 of the article. Individual observations are displayed as open dots that havebeen jittered vertically (panels a and b) and horizontally (panel b) to improve readability. 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 beforeforbs 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.

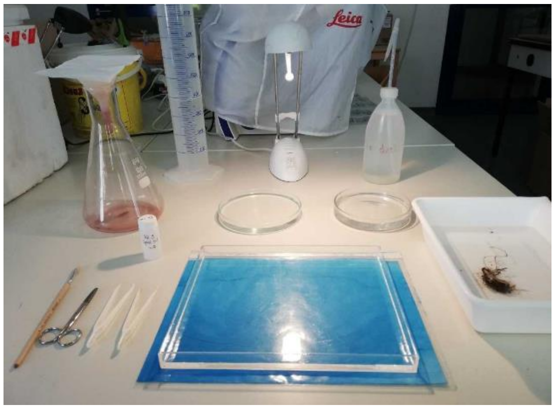


Supplementary Fig. 3. Root biomass distribution in different soil layers. For each treatment, mean values (solid dots) and compatibility intervals (solid bars) computed using non-parametric bootstrap are shown (*n*=5-7). Individual belowground observations are displayed as open dots. Sync1, all PFGs 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 beforeforbs and grasses; Sync2, all PFGs sown at the same time at the second sowing event.

3.2 Supplementary photographic material of Chapter 2

SEPTEMBER 2017
Rhizoboxes in the greenhouse.

SEPTEMBER 2020
Root growth in a rhizobox.



MAY 2020
Root cleaning

MAY 2020
Roots before and after being cleaned.

Chapter 3

Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment

Chapter 3

Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment

Article in preparation for submission

Inés M. Alonso-Crespo, Vicky M. Temperton, Andreas Fichtner, Michael Schloter, Silvia Gschwendtner and Benjamin M. Delory

Abstract

The order and timing of species immigration have an impact on species coexistence through priority effects driven by two main mechanisms: niche preemption and niche modification. Niche preemption restricts essential resources for later arrivals, whereas niche modification alters available niches through soil microbial and chemical legacies. The relative contributions of niche preemption and modification to the creation of priority effects have not yet been clearly measured.

In a plant–soil feedback experiment using soils from a POEM field experiment, we assessed the contributions of soil niche preemption and soil niche modification in generating priority effects in dry acidic grasslands above and below ground.

The results showed that soil niche preemption had the strongest effect at plant functional group (PFG) level when acting in combination with soil niche modification. Together, these mechanisms decreased plant productivity across all PFG. Moreover, soil niche modification alone did not produce significant effects in PFG above- or belowground productivity. However, at the species level, simultaneous occurrence of soil niche modification and soil niche preemption had a negative impact on the majority of the species affected, with forbs legacy being the most detrimental, especially for legumes. Additionally, soil niche modification alone increased belowground productivity of *Agrostis capillaris* and *Bromus hordeaceus.*

These findings indicate that the combination of resource uptake (soil niche preemption) and changes in soil microbial communities (soil niche modification) by early arriving species creates priority effects on plant productivity in dry grasslands.

Keywords: grasslands, nutrient depletion, microbial legacies, plant order of arrival, productivity, soil niche pre-emption, soil niche modification, species coexistence

Introduction

Priority effects occur when the order and timing of species immigration affect the coexistence of species due to early arrival species changing the environment and making it less or more suitable for later arriving species (Fukami, 2015). Priority effects can lead to changes in plant-plant interactions and hence affect both ecosystem functioning and community composition and diversity (Weidlich et al., 2018; Werner et al., 2020). The role of such legacy effects of order of arrival can also create alternative states in vegetation, and the importance of such historical contingencies in natural communities has recently begun to become more recognized (Grman & Suding, 2010). Fukami (2015), proposed two main classes of mechanisms behind priority effects: niche preemption and niche modification. Niche preemption occurs when early arriving species use available resources, such as nutrients, light, or space, reducing them for the later arriving species. Niche modification occurs when early arriving species change the types of niches available for later-arriving species, such as changing soil microbial communities or soil chemicals (including root exudates). Plant species condition the soil where they are established, changing biotic and abiotic environmental conditions that can influence the performance of other plants of the same or different species. This is commonly known as plant-soil feedback (PSF) (Bever et al., 1997; De Long et al., 2023; Ehrenfeld et al., 2005; Kulmatiski & Kardol, 2008; Kardol et al., 2015; van der Putten et al., 2013). Priority effect mechanisms can be included as plant-soil feedback processes because early arriving species preempt and condition the soil, and can thus affect the response of the development of later arriving species (De Long et al. 2023). Plant-soil feedback is driven by intrinsic or extrinsic, biotic and abiotic factors. (De Long et al. 2023).

Nutrient depletion always occurs in the soil when a species arrives first, throughout the niche preemption mechanism. Nutrient acquisition is an important process in plant development. Nutrient-rich environments can encourage plant species to develop more roots, and this can be modulated by species competition (Mommer et al. 2010, 2011, 2012). Macro-and micronutrients are essential for the correct development of plants. The presence of some micronutrients is related to the increased availability of macronutrients in the soil (Wang et al. 2022). In the absence or deficiency of these elements, plants can have a negative effect on their growth or performance (negative plant-soil feedback). Negative plant-soil feedback created by nutrient depletion is well known in the agricultural field as it causes a detrimental response in plant development. Cesarano et al. (2017) reviewed plant-soil feedback studies in agro- and natural ecosystems and found evidence of negative plant-soil feedbacks in both, proposing nutrient depletion as the primary causal factor of this negative response.

Niche modification can create alternative stages of the community influencing community structure and functioning merely by changing the different species that can be established under the new niche conditions (Fukami & Nakajima, 2011; Dickie et al. 2012).

Plants can modify soil conditions simply by being present. They interact with the environment, inducing changes that remain even when the originator plant is no longer present (Kulmatiski and Kardol 2008; van der Putten et al. 2013). Different plant communities generate different microbes (Heinen et al. 2020) and chemical legacies (Delory et al., 2021) that induce changes in the composition of subsequent plant communities. Root exudates and rhizosphere microbial communities are important drivers of temporal changes in the strength of the plant-soil feedback effects (Steinauer et al. 2023). These changes in microbial legacies usually occur when a species arrives first. The legacy of the soil microbiome is important for plant establishment. They also affect the growth and development of plants. Moreover, these legacies may still have an effect on plant-soil interactions by changing the composition of the endophytic community during the early stages of plant growth (Hannula et al. 2021). The impact of the microbial legacies of different species on other species has been studied, showing that, on average, all plant functional groups perform better when they grow in soil with microbial legacies from other species, which could indicate that pathogens are more plant-specific than plant growth-promoting biota.(Cortois et al. 2016).

Prior studies testing priority effects mechanisms have shown that niche preemption is more likely to be the major driver of priority effects when species arriving early and late have a great niche overlap or phylogenetic relationship (Tan et al. 2012; Vannette and Fukami 2014; Mwangi et al., 2007; Fargione, et al., 2003). Niche preemption is inhibitory and affects species within the niche (Fukami, 2015). In contrast, niche modification can be inhibitory or facilitative and can affect species across niches (Fukami, 2015). However, the contribution of each mechanism in creating priority effects is uncertain.

Through a plant-soil feedback experiment, we evaluated the extent to which soil niche preemption or modification drives the creation of priority effects above and belowground in a dry acidic grassland system. To test only the effects of soil niche preemption and soil niche modification, and not considering the effect of species competition for space or light, we isolated the two processes using solely the soil. We identified soil niche preemption with nutrient depletion on the substrate. For soil niche modification, we considered the changes in microbial communities that are commonly linked to the identity of the plant growing in the soil. The microbiome undergoes changes that are specific to the plants present in the community. We used a plant functional group (PFG) approach. The PFG consisted of species that shared common traits. Species with similar traits are expected to use the niche in a similar way. Using this approach, we aimed to increase the predictive power of the experiment. We conducted a plant-soil feedback experiment in two phases. The first phase was the conditioning phase, and we grew different plant community types for several weeks to alter the (a)biotic properties of the soil. The second phase was the feedback phase, designed as a response phase to test the following question:

- The relative contributions of soil niche preemption and soil modification to the creation of priority effects will depend on the PFG order of arrival. Specifically, we hypothesized that soil niche preemption will dominate when early- and late-arriving species belong to the same PFG. Soil niche modification, however, will dominate when early and late-arriving species belong to different PFG.

Material and Methods

To determine the extent to which the mechanisms of soil niche preemption and soil niche modification contribute to the creation of priority effects, we designed a plant–soil feedback experiment.

Soil collection and sterilization

The soil for all phases of the experiment was collected from the POEM experimental field site (see. Alonso-Crespo et al. submitted). The characteristics of the soil were typical of a dry acidic grassland (pH ($CaCl_2$) 4.9; organic matter content: 2.3%; total N: 0.07%; total C: 0.98%; C/N: 12.1). The soil was collected and brought to the Leuphana University Greenhouse in Lüneburg, Germany (53° 14′ 23.8″ N 10° 24′ 45.5″ E), and sieved at 5 mm to avoid large stones and dead roots.

The PSF experiment started in 2021 with a pre-experiment to test the nutrient flush effect in our soil (nutrient flush pre-experiment), but the main experiment, conditioning phase, and feedback phase were conducted in 2023. In all phases of the experiment, the soil was exposed to a minimum dose of 30 kGy of gamma radiation.

Species selection and sowing.

The selected plant species belonged to the dry acidic grassland species pool and were selected for their functional and morphological traits. We grouped them in three plant functional groups (PFG) and selected four species were selected per PFG: Grasses, species belonging to the *Poaceae* family, *Agrostis capillaris* (L.), *Anthoxanthum odoratum* (L.), *Bromus* *hordeaceus* (L.) and *Festuca ovina* (L.), Legumes, species members of *Fabaceae* family with the ability to fix atmospheric N_2, *Lathyrus pratensis* (L.), *Lotus corniculatus* (L.), *Trifolium arvense* (L.) and *Trifolium campestre* (Schreb.), and Forbs, containing the species that do not belong to the grasses or legumes groups, *Dianthus deltoides* (L.), *Pilosella officinarum* (L.), *Potentilla argentea* (L.) and *Silene vulgaris* [(Moench.) Garcke]. Seeds were obtained from Rieger-Hofmann GmbH (Blaufelden-Raboldshausen, Germany). Seeds for all phases of the experiment were sterilized following (Lindsey et al. 2017). For sowing, only one viable seed per species was left in each pot. Excedentary seedlings were removed to avoid confounding effects. Unsown species were weeded from pots during the entire experiment.

Nutrient flush pre-experiment

During soil sterilization, the availability of nutrients in the soil increases because of dead microorganisms and the release of nutrients. In order to test the effects of the nutrient flush of the sterilized soil in the development of the plants of our study, in January of 2021, a pre-experiment was conducted in a growth chamber at the Leuphana University of Lüneburg, Germany (53° 13’ 41.9″ N, 10° 24’ 11.0″ E). The soil was sent to BGS - Beta-Gamma-Service GmbH & Co. (Cologne, Germany) for gamma radiation. Two treatments were created: one with unsterilized live soil (U) (100%) and the second with a mixture of sterilized soil (S1) (90%) inoculated with unsterilized live soil (U) (10%). This second mixture of soil had a nutrient flush effect owing to sterilization. 32 pots (2 L) were filled with 1.300 kg of soil per treatment. To sow the pots, we created four combinations of three species belonging to the same PFG (forbs, grasses, or legumes), and each combination was replicated twice. Eight different mixtures composed by one legume, one grass, and one forb species were created. The experiment was running for 38 days and 1360 ml of water were added per pot during the experiment. The pots were randomly placed in trays, and their locations were randomized once per week during the entire experiment. The growth chamber was programmed with 24 h light owing to a programming error when setting up the environmental parameters of the growth chamber at the beginning of the experiment. The average temperature was 23.5 °C.

At the end of the experiment, plants were harvested at the species level. Roots were washed and collected at the community level. All materials were dried for 48h at 60 °C in a drying oven, and biomass information was obtained.

Plant-soil feedback experiment

Conditioning phase

In May 2022, the first batch of sieved (5 mm) soil was sent to Gammaton (Guanzate, Italy) for sterilization by gamma radiation.

The conditioning phase of the experiment was carried out under greenhouse conditions in Lüneburg, Germany (53° 14′ 23.8″ N, 10° 24′ 45.5″ E) from 18 of June 2022 to 13 of September 2022, with an average temperature of 20 °C. Sterilized soil (S) (90%) was inoculated with unsterilized live soil (U) (10%) to create a homogeneous soil mixture. A total of 384 pots (2 L) were filled with 2 kg of the soil mixture. During this phase, we created four soil legacies by manipulating the composition of the forbs, grasses, and legumes. The plant communities consisted of either three forb species (forb legacy), three grass species (grasses legacy), three legume species (legume legacy), or one species of each plant functional group (mix legacy) (Figure 1). Each plant community type was represented by four different species compositions, and pots were randomly placed over individual trays to avoid microbial contamination through water leaching on wheel-tables, which allowed their weekly randomization. The conditioning phase was conducted for eight weeks, and each pot received 1850 mL of water during the experiment. At the end of the conditioning phase, the plants were harvested at the species level. Before washing the roots, the soil was extracted to create the feedback-phase soil treatments. The soil was individually processed pot by pot. It was sieved to avoid the presence of root fragments from the plants growing in the conditioning phase in the soil of the feedback phase treatments. Roots from the plants growing in the conditioning phase were stored in bags at 4°C until they were washed. Root cleaning was performed for four weeks and were processed at the community level. Root and shoot samples were dried for 48h at 60 °C in a drying oven and weighed on an analytical scale. Pots in the conditioning phase were paired with the feedback phase pots; therefore, we had information about the soil legacy and the exact species composition that created it.

Feedback phase

In August 2022, a second batch of soil was sent to STERIS Synergy Health Radeberg GmbH (Radeberg, Germany) for sterilization by gamma radiation. To set up the feedback phase, we used soil conditioned during the conditioning phase (C), sterilized soil (S), and unsterilized live soil (U) to create three different soil treatments. The first soil treatment consisted in sterilized soil (S) (90%) inoculated with unsterilized live soil (10%), as the soil mixture used at the beginning of the conditioning phase. This established our control, where no soil preemption or soil modification occurred in the soil. The second soil treatment consisted of soil collected from the conditioning soil (C) (100%), in which we had soil niche preemption because of nutrient depletion, and soil niche modification because of microbial legacies. The third soil treatment was created by mixing sterilized soil (S) (90%) with an inoculum of conditioned live soil (10%) (C) from the soil obtained in the conditioning phase. This soil treatment corresponded with a no-soil niche preemption situation with soil niche modification because we added a microbial inoculum from the microbial soil legacies created in the conditioning phase to a

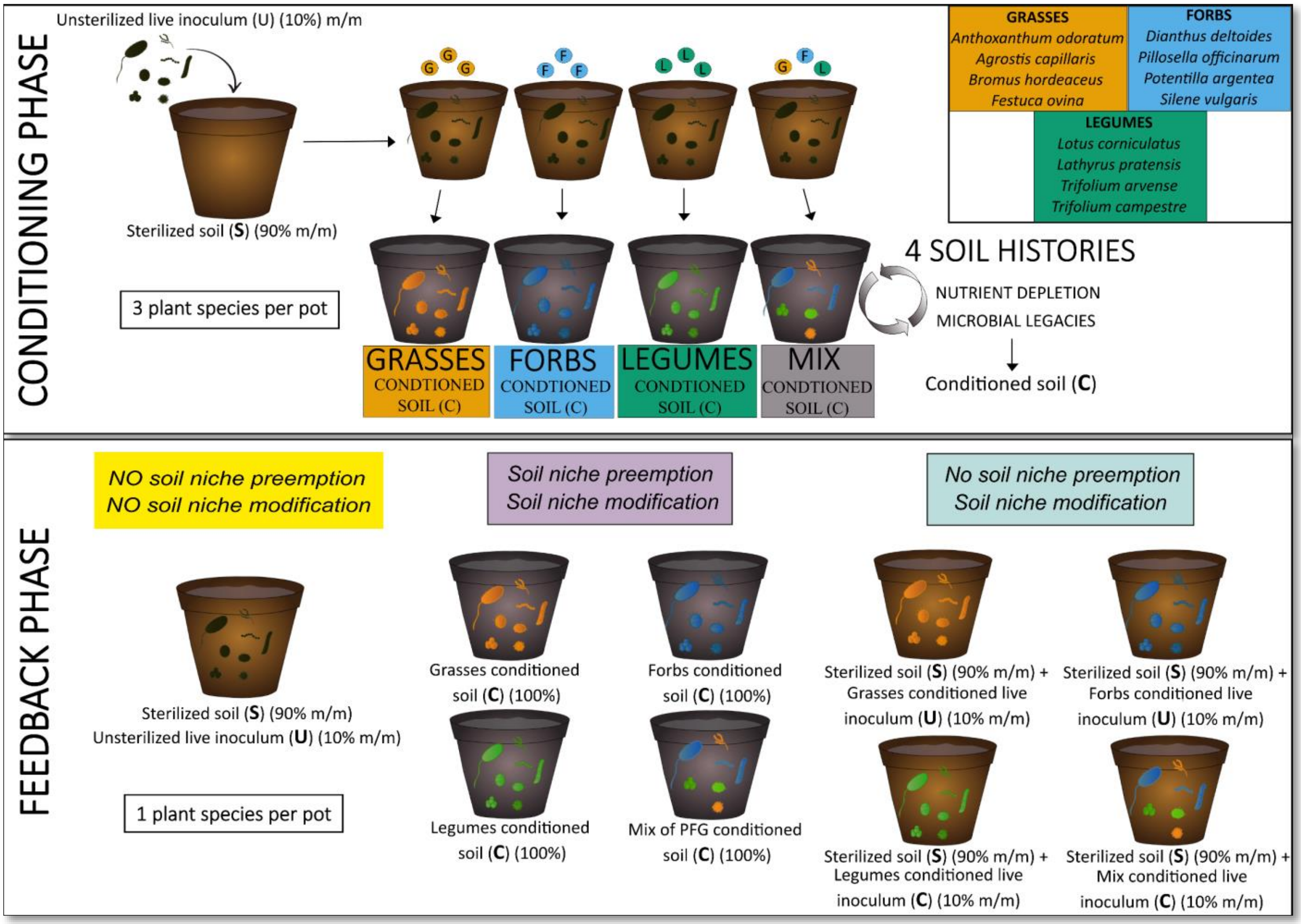


Figure 1. Experimental design of the conditioning and feedback phases.

sterilize soil (Figure 1). The feedback phase had 432 pots (1 L), and each pot had 1.200 kg of soil mixture. Pots were randomly placed over individual trays in wheel tables, which allowed weekly randomization. Only one individual per plant species was grown per pot and each species had four replicates per soil treatment. This phase was conducted for seven weeks as of 19th of September, with an average temperature of 16 °C. Each pot received 810 mL water during the experiment. Plants were harvested, and pots containing the soil and roots were stored at 4 °C until they were washed. Root cleaning was carried out for three and a half weeks. The shoot and root samples were dried for 48 h at 60 °C in a drying oven and weighed on an analytical scale.

Statistical analysis

In order to test the effect of the legacy (i.e., the soil history) and the treatment (i.e., soil type during the feedback phase) over plant functional group productivity we used a generalised linear-mixed-effect model (*GLMER*). The fixed effect considered was an aggregate variable Legacy_Treatment, with information from the soil history (grasses legacy, forbs legacy, legumes legacy and mix legacy) and the soil treatment (No: control – no soil niche preemption and no soil niche modification, NP+NM - soil niche preemption+soil niche modification - and NM - soil niche modification). Two random effects were considered, the identity of the species sown in the feedback phase and the species identity that conformed the legacies. The response variables were shoot dry mass or root dry mass (g) of the PFG. The model was fitted using a g*amma* distribution with a *log-link* function and a *bobyqa* optimizer using the *lm4* R package (Bates et al., 2015). To address the effect of the legacy and the treatment over individual plant species productivity we used a generalised linear model (*GLM*). The predictor variable considered was also the aggregate variable Legacy_Treatment used for the *GLMER*, with the same levels. The response variable was shoot dry mass (g) or root dry mass (g) of the species. The model was fitted using a *gamma* distribution with a *log-link* function with the *MASS* R package (Ripley et al. 2013). After GLMER and GLM linear models were fitted, an analysis of variance (ANOVA) was performed on both for assessing the significance of different predictor variables using the function *ANOVA* from the R package car (Fox J, Weisberg S, 2019). Post-hoc tests were conducted using *emmeans* R package (Lenth, 2023).

F.ovina seeds were contaminanted with seeds of *Poa trivialis* (L.). We decided to keep all the pots were they were growing for the PFG analysis because both were grasses, but we excluded them from the species analysis.

The strength of the biological effects was quantified using relative effect sizes (%). The control situation (no soil preemption and no soil niche modification) was used as a reference for calculating the relative effect sizes.

Data analysis were performed in R version 4.3.0 (R Core Team, 2023). Additionally, these R packages were used as well for data exploration, visualization, and analysis: R packages included in *tidyverse* (Wickham et al., 2019), *ggpubr* (Kassambara, 2023), *ggplot2* (Wickham 2016) and *viridis* (Garnier et al., 2021).

Results

Nutrient flush

The increase in nutrient availability during the soil sterilization process was found to affect the shoot productivity of all PFG. Shoot productivity was higher for all PFG when grown in sterilized soil (with a nutrient flush effect) than in unsterilized live soil. Root productivity in grasses seems to be more affected by soil sterilisation than the other PFG (Figure S1).

Conditioning phase

At the end of the conditioning phase, the productivity of all the plant communities that created the four soil histories was measured. The total, shoot, and root biomass were lower when the community was formed by legume species (Figure 2).

Feedback phase

Soil niche modification and soil niche preemption created priority effects when acted together. Soil niche modification alone (microbial legacies) did not affect total, shoot, or root biomass. Shoot biomass of all PFGs was reduced when they grew on soils conditioned by a mixture of PFG, grasses, or forbs. Legumes shoot biomass was also reduced in soil conditioned by legumes. (Figure 3, Figure S2, and Table 1). In the case of root dry weight, legumes and forbs reduced their biomass when grew in soil conditioned by a mixture of PFG and by forbs. Forbs root biomass was also reduced in soil conditioned by grasses (Figure 3, Figure S3, and Table 1). The root mass fraction was higher for grasses growing in soil conditioned by grasses (Figure S4).

At the species level, soil niche modification did not significantly affect shoot development (Figure S5 and Table 1). In contrast, it affected the root productivity of two grass species (Figure. S6 and Table 1). *Agrostis capillaris* benefited significantly by growing in soil inoculated with microbial legacies of forbs (+171), grasses (+143%) and legumes (+150%) and *Bromus hordeaceus* by growing in soil inoculated with microbial legacies of forbs (+210%) and legumes (+153%). Similar to the PFG level, at the species level, soil niche preemption and soil niche modification together affected shoot and root productivity. Shoot productivity was reduced in soil conditioned by forbs for *Dianthus deltoides* (-56%), *Potentilla argentea* (-74%), and the four legumes species, *Lathyrus pratensis* (-64%), *Lotus corniculatus* (-76%), *Trifolium arvense* (-75%), and *Trifolium campestre* (-73%). Soil conditioned by grasses affected shoot productivity of *Dianthus deltoides* (-76%), *Silene vulgaris* (-62%), and *Potentilla argentea* (-8%). Soil conditioned by mix of

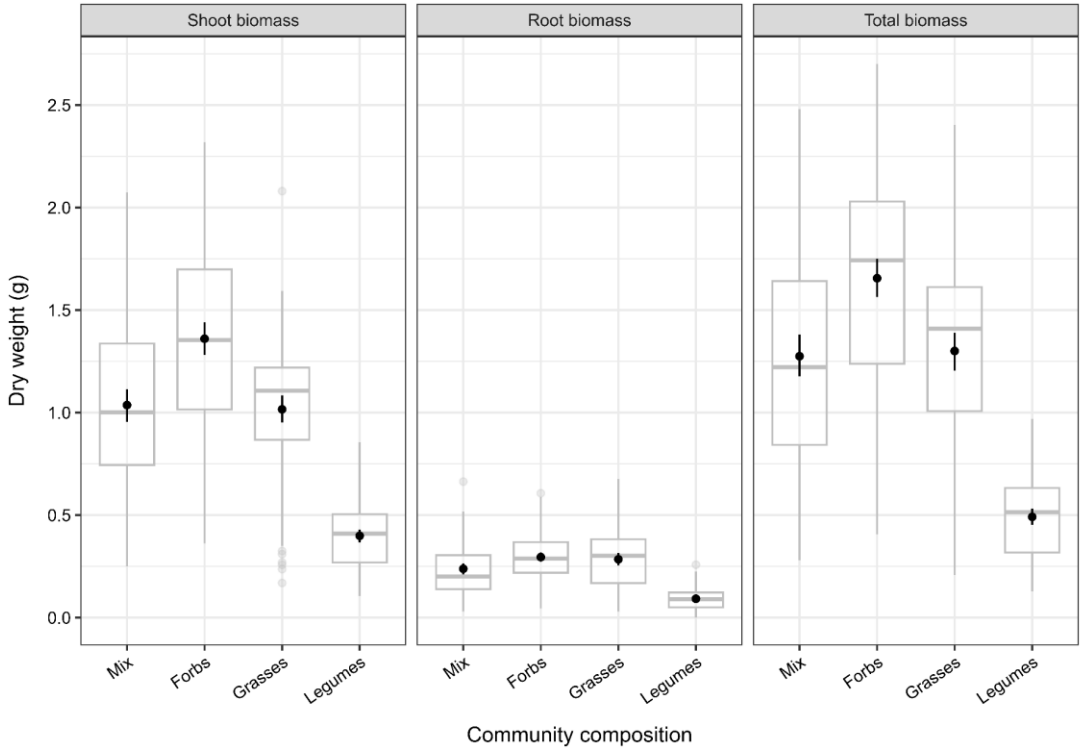


Figure 2. Shoot dry weight (g), root dry weight (g), and total dry weight (g) of the plant communities at the end of the conditioning phase. On the x-axis, the plant communities that created the soil histories (legacies) are represented as follows: mix (all three functional groups with one species per group), forbs (three species of forbs), grasses (three species of grasses), and legumes (three species of legumes). Black closed dots over the boxplots represent the average value of the group, and a 95% CI calculated using non-parametric bootstrap is shown.

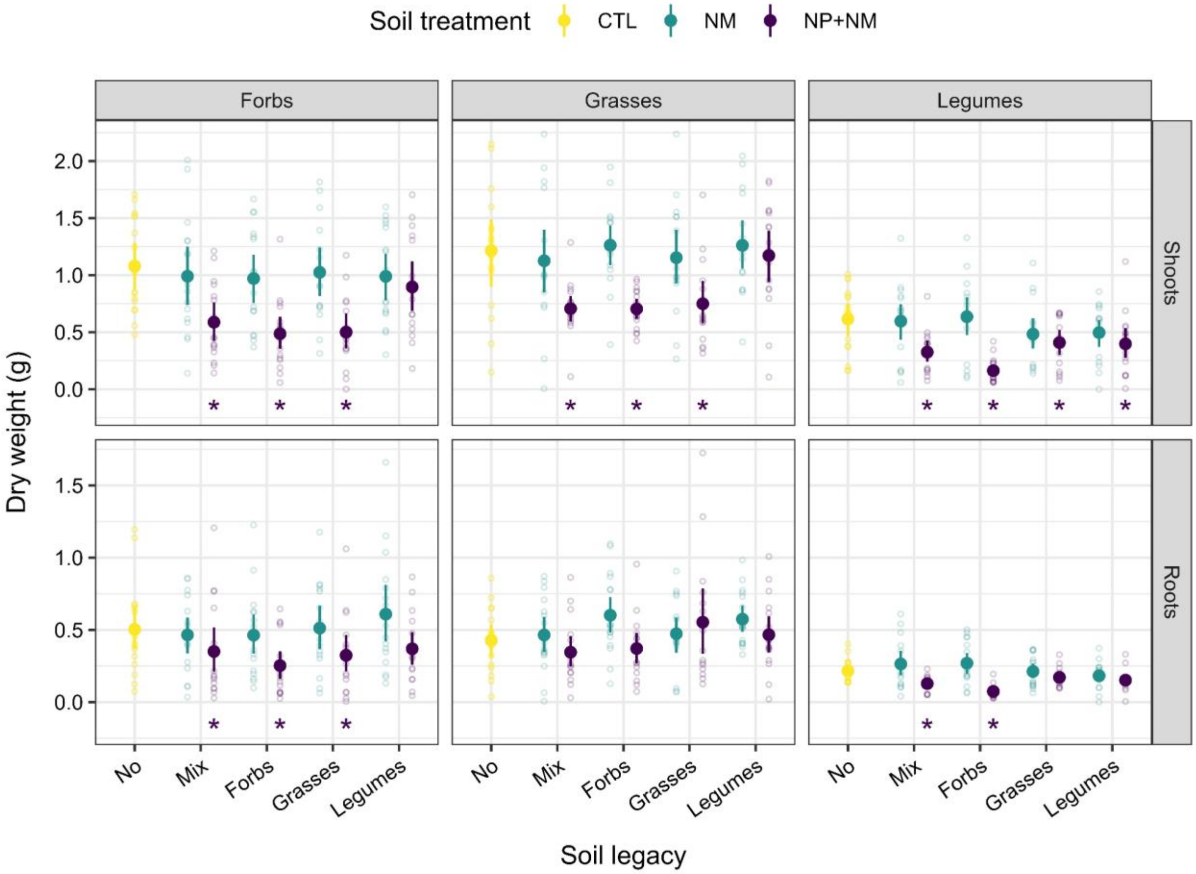


Figure 3 Shoot dry weight (g) and root dry weight (g) of forbs, grasses, and legumes, under control (yellow), soil niche modification (blue), or soil niche modification + soil niche preemption (purple) soil conditions. Forbs, grasses, and legumes on the top of the graph represent the PGF to which the plant species sown in the feedback phase belonged. Closed dots represent the mean value, and 95% confidence interval computed using non-parametric bootstrap are shown (n=45-48). Open dots represent the observed shoot or root dry weight values, which were jittered horizontally to improve readability. On the x-axis, the soil legacies created in the conditioned soil are represented as follows: No: No legacy (this is our control), Mix: mix of PFG legacy, Forbs: forbs species legacy, Grasses: grasses species legacy, and Legumes, legume species legacy. Groups marked with an asterisk are significantly different from the no legacy control (yellow group).

PFG reduced shoot productivity of *Potentilla argentea* (-59%), *Pillosella officinarum* (-51%), and *Lotus corniculatus* (-55%) (Figure S5). Root productivity of *Potentilla argentea* (-71%), *Dianthus deltoides* (-58%), and the four legume species, Lathyrus pratensis (-58%), *Lotus corniculatus* (-70%), *Trifolium arvense* (-71%), and *Trifolium campestre* (-59%) were also reduced growing in soil conditioned by forbs. *Bromus hordeaceus* increased root productivity (+244%), and *Dianthus deltoides* diminished it (-78%) in soil conditioned by grasses. *Potentilla argentea* reduced root productivity (-58%) growing in soil conditioned by mix (Figure S6 and Table 1).

Discussion

Here, we showed that soil niche modification alone does not affect the plant development at plant functional group level; it was in combination with soil niche preemption, where both together reduced root

PFG	Soil niche modification								Soil niche preemption + Soil niche modification								
	Legacy								Legacy								
	Grasses		Forbs		Legumes		Mix		Grasses			Forbs		Legumes		Mix	
	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW	RMF	SDW	RDW	SDW	RDW	SDW	RDW
Grasses									-38%		54%	-42%				-42%	
Forbs									-54%	-36%		-55%	-50%			-46%	-30%
Legumes									-34%			-73%	-66%	-35%		-47%	-41%

Species	Soil niche modification								Soil niche preemption + Soil niche modification							
	Legacy								Legacy							
	Grasses		Forbs		Legumes		Mix		Grasses		Forbs		Legumes		Mix	
	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW
Agrostis capillaris		143%		171%		150%										
Anthoxanthum hodorathum																
Bromus hordeaceus				210%		153%				244%						
Dianthus deltoides									-76%	-78%	-56%	-58%				
Silene vulgaris									-62%							
Pillosella officinarum															-51%	
Potentilla argentea									-8%		-74%	-71%			-59%	-58%
Lathyrus pratensis											-64%	-58%				
Lotus corniculatus											-76%	-70%			-55%	
Trifolium arvense											-75%	-71%				
Trifolium campestre											-73%	-59%				

Table 1. Relative effect sizes of soil niche modification and soil niche preemption + soil niche modification treatment and soil legacy at plant functional group and species level.

and shoot productivity. However, at the species level, we detected some species-specific effects of soil niche modification on root productivity and a mainly negative effect of the combination of soil niche preemption and soil niche modification on the above- or belowground productivity depending on the species.

Contrary to our hypothesis, soil niche modification did not affect plant development at the PFG level in any scenario. Even when the PFG responsible for creating the legacy in the conditioning phase was the same or different from the PFG in the feedback phase, there was no observable effect of soil niche modification. However, soil niche preemption and soil niche modification together affected plant development at the PFG level. The combination of soil niche preemption and soil niche modification always showed negative response. Although the PFG responded to their own legacy, it was the forbs' legacy the most detrimental one. Changes in nutrient availability and soil microbial legacies affected the development of later-arriving PFG. Community composition in natural grassland systems can be shaped by the conjunction of niche preemption and niche modification, because once a species is established, its legacy would endure for a long time, affecting assembly outcomes (Helsen et al., 2016). At the plant functional group level, in all cases where our results indicated an effect of soil niche preemption and soil niche modification treatment this was negative, reducing shoot and root biomass. These findings showed that during the feedback phase, forbs, grasses, and legumes suffered a decrease in their shoot productivity growing in soil conditioned by mix, forbs and grasses, with legumes being the only PFG affected by soil conditioned by itself. Root productivity was reduced for forbs growing in soil conditioned by forbs, grasses, and mix, and legumes in soil conditioned by mix and forbs. Grasses was the only PFG that was not affected by any legacy on root productivity. In addition, its root mass fraction was positively affected by its own legacy, which could indicate belowground intra- or interspecific competition with grasses species. Legumes was the only legacy that did not affect the other PFG. In the case of legumes, an increase of intra- or interspecific competition with other legumes species, with the reduction in specific nutrients might be a possible explanation for the lower legumes' performance in their own soil legacy. Forbs intervened in the creation of forbs and mix of PFG legacies during the conditioning phase. This, might have given them the advantage of take up some essential nutrients for plant development which could have impact shoot productivity of all the PFG, but also the root productivity of forbs and legumes. Forbs are highly dependent on the K content (Tilman et al., 1999), growing in a soil with deficit in that nutrient would be detrimental for its own or others development. Grasses intervened in the creation of the grasses and mix of PFG legacies during the conditioning phase, which could have implied a decrease in the total nutrient levels, as they are high competitors (Linder et al., 2018), which could explain the reduced shoot productivity of all PFG growing in soil conditioning by them or the reduction in root productivity in the case of forbs. Nutrient depletion by early arrival species seems to be the principal driver, since soil niche modification alone did significantly affect any of the PFG development. Further investigation is needed at the microbiome level to determine whether the absence of the impact of soil niche modification on the PSF response is a consequence of the lack of sufficient species-specific microorganisms. The inoculums of the soil niche modification treatment in the feedback phase represented only 10% of the soil weight, with the other 90% being sterilized soil. This could have caused a significant reduction in the presence of specific microbial groups. Soil type has been shown to be one of the main drivers of microbial community promotion (Philippot et al. 2013). Moreover, previous studies have shown that in sandy soils, the plant-soil-feedback responses are caused by abiotic soil conditions and by plant-soil microbial legacies (Bezemer et al., 2006). The soil used in this study is not only a sandy soil, but also a nutrient poor with acid pH, parameters which can alter PSF responses (Ehrenfeld et al., 2005). These characteristics could lead to a limiting environment in which nutrient acquisition is the main limiting factor for plant development, not only at the PFG level, but also at the species level. Contrary to our findings, previous studies have demonstrated the effects of plant microbial legacies on subsequent plant species (Cortois et al. 2016; Semchenko et al. 2019; Heinen et al. 2020; Hannula et al. 2021). The soil in these studies differs among them, but all have higher pH and nutrient levels than ours. This could be the reason why our results did not detect the effect of microbial legacies at the PFG level. Research investigating how soil properties can impact PSF responses is needed to determine if some of these parameters are decisive in the stimulation of specific microbial communities or if the limitation of the soil environment determines the strength of the PSF.

When we took a closer look at the species level, soil niche modification resulted in a positive response of two of the species to specific legacies in root development. Forbs, grasses, and legumes microbial legacies increased *A. capillaris* and forbs, and legumes microbial legacies increased *B. hordeaceus* root biomass. Our findings suggest that soil niche modification effects on plant growth are species-specific and depend on the legacy type. Šmilauer and Šmilauerová (2013) observed a similar response in a soil nutrient depletion context, with grasses species benefiting from the presence of legumes and forbs species. Intra- and inter-specific legacies can determine the establishment of species, driving the community composition (van de Voorde et al., 2011). Negative PSF have been detected from different ratios of grasses and forbs over species of their same PFG (Heinen et al. 2020), and positive PSF have been detected for species growing in soil conditioned by other species (Cortois et al. 2016; Huberty et al. 2022; Zhang et al., 2022). Intraspecific legacies appear to be more likely negative than interspecific ones (Kulmatiski and Kardol 2008; Hendriks et al. 2013), as we see in this case, where two grasses benefited from legumes and forbs legacies. However, Delory et al., (2021) demonstrated that different plant functional groups generate distinct metabolomes creating specific soil chemical legacies that affect later-arrival species differently depending on their identity. The observed response of *A.capillaris* and *B. hordeaceus* to specific soil legacies could have been also motivated by soil chemical legacies, and not only by microbial legacies. Additional information is required to determine whether these changes are due to soil microbial or soil chemical legacies.

Plant development responded to the combined effects of soil niche preemption and soil niche modification at the species level. These responses varied with species identity, affecting in more or less degree the growth of the plants depending on it, but mainly negatively. The legacy of forbs was prejudicial for more than half of the later arriving species, affecting the overall legume species or other forbs. As previously mentioned, the uptake of specific nutrients by forbs (Tilman et al., 1999) could be the reason to explain this negative effect of the forbs soil legacy at species level, specially over legumes, which seems to be a plant functional group very sensitive to soil environmental changes since they were affected by every soil legacy at the PFG level. Growing in soil conditioned by grasses diminished the shoot growth of *S. vulgaris,* possibly due to nutrient depletion. In the case of *D. deltoides,* shoot and root growth were reduced. This species has been previously found to react negatively to grass and forbs legacies, specifically to their soil chemical legacies (Delory et al., 2021). Grasses legacy was the only one which generated a positive response. This occurred with *B.hordeaceus,* which produced more roots in grass-conditioned soil. Surprisingly, this species did not benefit from arriving earlier in the soil niche modification treatment under grasses legacy, so it is unlikely that microbial legacies are responsible for this event. *D. deltoides, P. argentea*, *P. officinarum*, and *L. corniculatus* reduced their growth above- or belowground, or in both cases, growing in soil conditioned by a mix of PFG. With the exception of *P. officinarum*, these species were also affected by forbs or grasses legacies. Both PFG conform the mix of PFG legacy together with legumes, which could trigger the same effects over these species in the mix than the forbs or grasses legacies.

In conclusion, soil niche preemption and soil niche modification acting together created priority effects in dry acidic grassland plant communities. However, soil niche preemption appears to be the major driver in the creation of these priority effects under nutrient-poor soil characteristics. Further investigation is needed to clarify if soil properties and nutrient levels can determine the impact of microbial legacies on plant communities. Understanding the consequences of different soil systems in the creation of priority effects via soil niche preemption, soil niche modification or their combination will help to comprehend different paths of species coexistence and community trajectories.

References

Alonso-Crespo, Inés María, Temperton, Vicky M., Fichtner, Andreas, Niemeyer, Thomas Schloter, Michael, Delory, Benjamin M. Submitted. “Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment”. *Journal of Ecology. ID JEcol-2023-1025.*

Bates, Douglas, Martin Maechler, Ben Bolker, and Steve Walker. 2015. “Fitting Linear Mixed-Effects Models Using Lme4.” *Journal of Statistical Software* 67 (1): 1–48.

Bever, James D., Kristi M. Westover, and Janis Antonovics. 1997. “Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach.” *The Journal of Ecology* 85 (5): 561–73.

Bezemer, T. Martijn, Clare S. Lawson, Katarina Hedlund, Andrew R. Edwards, Alex J. Brook, José M. Igual, Simon R. Mortimer, and Wim H. Van Der Putten. 2006. “Plant Species and Functional Group Effects on Abiotic and Microbial Soil Properties and Plant-Soil Feedback Responses in Two

Grasslands." *The Journal of Ecology* 94 (5): 893–904.

Cesarano, G., M. Zotti, V. Antignani, R. Marra, F. Scala, and G. Bonanomi. 2017. "Soil sickness and negative plant-soil feedback: a reappraisal of hypotheses." *Journal of Plant Pathology: An International Journal of the Italian Phytopathological Society* 99 (3): 545–70.

Cortois, Roeland, Thomas Schröder-Georgi, Alexandra Weigelt, Wim H. Putten, and Gerlinde B. De Deyn. 2016. "Plant–Soil Feedbacks: Role of Plant Functional Group and Plant Traits." *The Journal of Ecology* 104 (6): 1608–17.

De Long, Jonathan R., Robin Heinen, Johannes Heinze, Elly Morriën, G. Kenny Png, Sarah J. Sapsford, François P. Teste, and Ellen L. Fry. 2023. "Plant-Soil Feedback: Incorporating Untested Influential Drivers and Reconciling Terminology." *Plant and Soil*, 1-37.

Delory, Benjamin M., Hannes Schempp, Sina Maria Spachmann, Laura Störzer, Nicole M. van Dam, Vicky M. Temperton, and Alexander Weinhold. 2021. "Soil Chemical Legacies Trigger Species-specific and Context-dependent Root Responses in Later Arriving Plants." *Plant, Cell & Environment*, February, ce.13999.

Dickie, Ian A., Tadashi Fukami, J. Paula Wilkie, Robert B. Allen, and Peter K. Buchanan. 2012. "Do Assembly History Effects Attenuate from Species to Ecosystem Properties? A Field Test with Wood-Inhabiting Fungi." *Ecology Letters* 15 (2): 133–41.

Ehrenfeld, Joan G., Beth Ravit, and Kenneth Elgersma. 2005. "FEEDBACK IN THE PLANT-SOIL SYSTEM." *Annual Review of Environment and Resources* 30 (1): 75–115.

Fox J, Weisberg S. 2019. *An R Companion to Applied Regression*. Sage, Thousand Oaks CA.

Fukami, Tadashi. 2015. "Historical Contingency in Community Assembly : Integrating Niches, Species Pools, and Priority Effects." *Annual Review of Ecology, Evolution, and Systematics* 46: 1–23.

Fukami, Tadashi, and Mifuyu Nakajima. 2011. "Community Assembly: Alternative Stable States or Alternative Transient States?" *Ecology Letters* 14 (10): 973–84.

Garnier, S., N. Ross, R. Rudis, A. P. Camargo, and M. Sciaini. 2021. "Rvision-Colorblind-Friendly Color Maps for R." *R Package Version 0.6*.

Grman, Emily, and Katharine N. Suding. 2010. "Within-Year Soil Legacies Contribute to Strong Priority Effects of Exotics on Native California Grassland Communities." *Restoration Ecology* 18 (5): 664–70.

Hannula, S. Emilia, Robin Heinen, Martine Huberty, Katja Steinauer, Jonathan R. De Long, Renske Jongen, and T. Martijn Bezemer. 2021. "Persistence of Plant-Mediated Microbial Soil Legacy Effects in Soil and inside Roots." *Nature Communications* 12 (1): 5686.

Heinen, Robin, S. Emilia Hannula, Jonathan R. De Long, Martine Huberty, Renske Jongen, Anna Kielak, Katja Steinauer, Feng Zhu, and T. Martijn Bezemer. 2020. "Plant Community Composition Steers Grassland Vegetation via Soil Legacy Effects." *Ecology Letters* 23 (6): 973–82.

Helsen, Kenny, Martin Hermy, and Olivier Honnay. 2016. "A Test of Priority Effect Persistence in Semi-Natural Grasslands through the Removal of Plant Functional Groups during Community Assembly." *BMC Ecology* 16 (April): 22.

Hendriks, Marloes, Liesje Mommer, Hannie de Caluwe, Annemiek E. Smit-Tiekstra, Wim H. van der Putten, and Hans de Kroon. 2013. "Independent Variations of Plant and Soil Mixtures Reveal Soil Feedback Effects on Plant Community Overyielding." *The Journal of Ecology* 101 (2): 287–97.

Huangfu, Chaohe, Liming Zhang, and Dafeng Hui. 2022. "Density-Dependent Plant-Soil Feedbacks of Two Plant Species Affected by Plant Competition." *The Science of the Total Environment* 807 (Pt 2): 150908.

Huberty, Martine, Katja Steinauer, Robin Heinen, Renske Jongen, S. Emilia Hannula, Young Hae Choi, and T. Martijn Bezemer. 2022. "Temporal Changes in Plant Soil Feedback Effects on Microbial Networks, Leaf Metabolomics and Plant-insect Interactions." *The Journal of Ecology*, 110(6), 1328-1343.

Kassambara, Alboukadel. 2023. “Ggpubr: ‘ggplot2’ Based Publication Ready Plots. R Package Version 0.6.0.” Comprehensive R Archive Network (CRAN). February 10, 2023. https://CRAN.R-project.org/package=ggpubr.

Kulmatiski, Andrew, and Paul Kardol. 2008. “Getting Plant—Soil Feedbacks out of the Greenhouse: Experimental and Conceptual Approaches.” In *Progress in Botany*, edited by Ulrich Lüttge, Wolfram Beyschlag, and Jin Murata, 449–72. Berlin, Heidelberg: Springer Berlin Heidelberg.

Lenth, Russell V. 2023. “Estimated Marginal Means, Aka Least-Squares Means [R Package Emmeans Version 1.8.8],” August. https://CRAN.R-project.org/package=emmeans.

Linder, H. P., Caroline E. R. Lehmann, Sally Archibald, Colin P. Osborne, and David M. Richardson. 2018. “Global Grass (Poaceae) Success Underpinned by Traits Facilitating Colonization, Persistence and Habitat Transformation.” *Biological Reviews of the Cambridge Philosophical Society* 93 (2): 1125–44.

Lindsey, Benson E., 3rd, Luz Rivero, Chistopher S. Calhoun, Erich Grotewold, and Jelena Brkljacic. 2017. “Standardized Method for High-Throughput Sterilization of Arabidopsis Seeds.” *Journal of Visualized Experiments: JoVE*, no. 128

Mommer, Liesje, Jasper van Ruijven, Hannie De Caluwe, Annemiek E. Smit-Tiekstra, Cornelis A. M. Wagemaker, N. Joop Ouborg, Gerard M. Bögemann, Gerard M. Van Der Weerden, Frank Berendse, and Hans de Kroon. 2010. “Unveiling Below-Ground Species Abundance in a Biodiversity Experiment: A Test of Vertical Niche Differentiation among Grassland Species.” *The Journal of Ecology* 98 (5): 1117–27.

Mommer, Liesje, Jasper van Ruijven, Corien Jansen, Harry M. van de Steeg, and Hans de Kroon. 2012. “Interactive Effects of Nutrient Heterogeneity and Competition: Implications for Root Foraging Theory?” *Functional Ecology* 26 (1): 66–73.

Mommer, Liesje, Eric J. W. Visser, Jasper van Ruijven, Hannie de Caluwe, Ronald Pierik, and Hans de Kroon. 2011. “Contrasting Root Behaviour in Two Grass Species: A Test of Functionality in Dynamic Heterogeneous Conditions.” *Plant and Soil* 344 (1): 347–60.

Paul Kardol, G. F. (Ciska) Veen, François P. Teste, and Michael P. Perring. 2015. “Peeking into the Black Box: A Traitbased Approach to Predicting Plant–Soil Feedback.” *The New Phytologist* 206 (1): 1–4.

Philippot, Laurent, Jos M. Raaijmakers, Philippe Lemanceau, and Wim H. van der Putten. 2013. “Going Back to the Roots: The Microbial Ecology of the Rhizosphere.” *Nature Reviews. Microbiology* 11 (11): 789–99.

Putten, Wim H. van der, Richard D. Bardgett, James D. Bever, T. Martijn Bezemer, Brenda B. Casper, Tadashi Fukami, Paul Kardol, et al. 2013. “Plant-Soil Feedbacks: The Past, the Present and Future Challenges.” *The Journal of Ecology* 101 (2): 265–76.

R Core Team. 2023. “R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL Http://www.r-project.org/.” Vienna: R Foundation for Statistical Computing. https://www.R-project.org/.

Ripley, Brian, Bill Venables, Douglas M. Bates, Kurt Hornik, Albrecht Gebhardt, David Firth, and Maintainer Brian Ripley. 2013. “Package ‘Mass.’” *Cran r* 538: 113–20.

Semchenko, Marina, Jonathan W. Leff, Yudi M. Lozano, Sirgi Saar, John Davison, Anna Wilkinson, Benjamin G. Jackson, et al. 2019. “Fungal Diversity Regulates Plant-Soil Feedbacks in Temperate Grassland.” *Science Advances* 4 (11): eaau4578.

Šmilauer, P., and M. Šmilauerová. 2013. “Asymmetric Relationship between Grasses and Forbs: Results from a Field Experiment under Nutrient Limitation.” *Grass and Forage Science* 68 (1): 186–98.

Steinauer, Katja, Madhav P. Thakur, S. Emilia Hannula, Alexander Weinhold, Henriette Uthe, Nicole M. van Dam, and T. Martijn Bezemer. 2023. “Root Exudates and Rhizosphere Microbiomes Jointly Determine Temporal Shifts in Plant-Soil Feedbacks.” *Plant, Cell & Environment*,

Tan, Jiaq, Zhichao Pu, Wade A. Ryberg, and Lin Jiang. 2012. “Species Phylogenetic

Relatedness, Priority Effects, and Ecosystem Functioning." *Ecology* 93 (5): 1164–72.

Tilman, Elizabeth A., David Tilman, Michael J. Crawley, and A. E. Johnston. 1999. "Biological Weed Control via Nutrient Competition: Potassium Limitation of Dandelions." *Ecological Applications: A Publication of the Ecological Society of America* 9 (1): 103–11.

Vannette, Rachel L., and Tadashi Fukami. 2014. "Historical Contingency in Species Interactions: Towards Niche-Based Predictions." *Ecology Letters* 17 (1): 115–24.

Voorde, Tess F. J. van de, Wim H. van der Putten, and T. Martijn Bezemer. 2011. "Intra- and Interspecific Plant-Soil Interactions, Soil Legacies and Priority Effects during Old-Field Succession." *The Journal of Ecology* 99 (4): 945–53.

Wang, Zhaoyu, Yongzhen Ding, Xiuna Ren, Jianwen Xie, Sunil Kumar, Zengqiang Zhang, and Quan Wang. 2022. "Effect of Micronutrient Selenium on Greenhouse Gas Emissions and Related Functional Genes during Goat Manure Composting." *Bioresource Technology* 349 (April): 126805.

Weidlich, Emanuela W. A., Philipp von Gillhaussen, Johannes F. J. Max, Benjamin M. Delory, Nicolai D. Jablonowski, Uwe Rascher, and Vicky M. Temperton. 2018. "Priority Effects Caused by Plant Order of Arrival Affect Below-ground Productivity." *The Journal of Ecology* 106 (2): 774–80.

Werner, Chhaya M., Katharine L. Stuble, Anna M. Groves, and Truman P. Young. 2020. "Year Effects: Interannual Variation as a Driver of Community Assembly Dynamics." *Ecology* 101 (9): e03104.

Wickham, Hadley. 2016. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wickham, Hadley, Mara Averick, Jennifer Bryan, Winston Chang, Lucy McGowan, Romain François, Garrett Grolemund, et al. 2019. "Welcome to the Tidyverse." *Journal of Open Source Software* 4 (43): 1686.

4.1 Supplementary information of Chapter 3

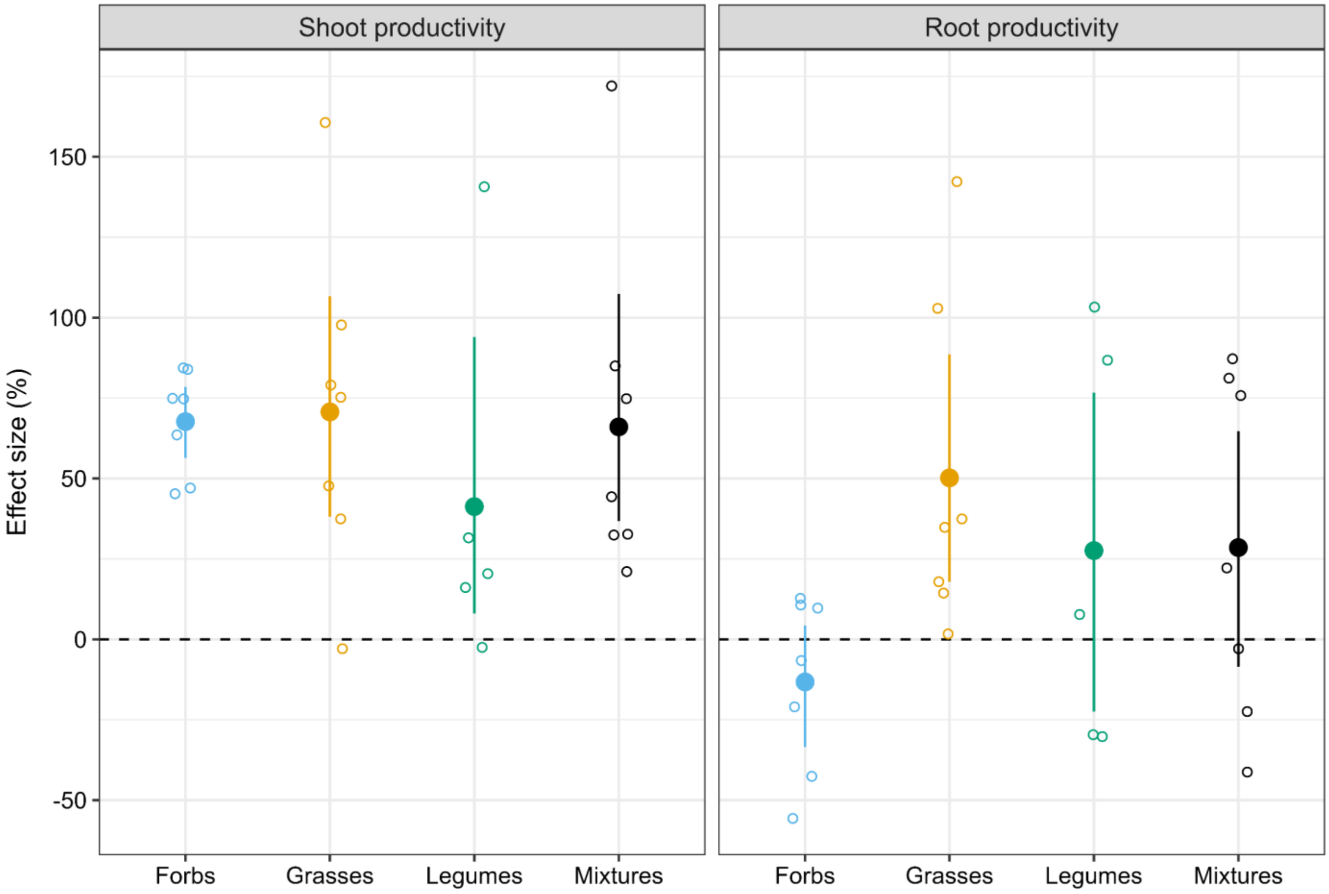


Figure S1. Relative effect size of shoot and root productivity of forbs, grasses, legumes, and mixtures of PFG communities in sterilized soil treatments compared to the control (unsterilized). The dashed-line represent the zero value. Closed dots represent the average value of the effect sizes of the communities growing in a mixture of sterilized soil (S1) (90%) + inoculated with unsterilized live soil (U) (10%), with nutrient flush, and 95% confidence intervals computed using non-parametric bootstrap are shown (n=5-7). Open dots represent the relative effect sizes for shoot and root dry weight values, which were jittered horizontally to improve readability.

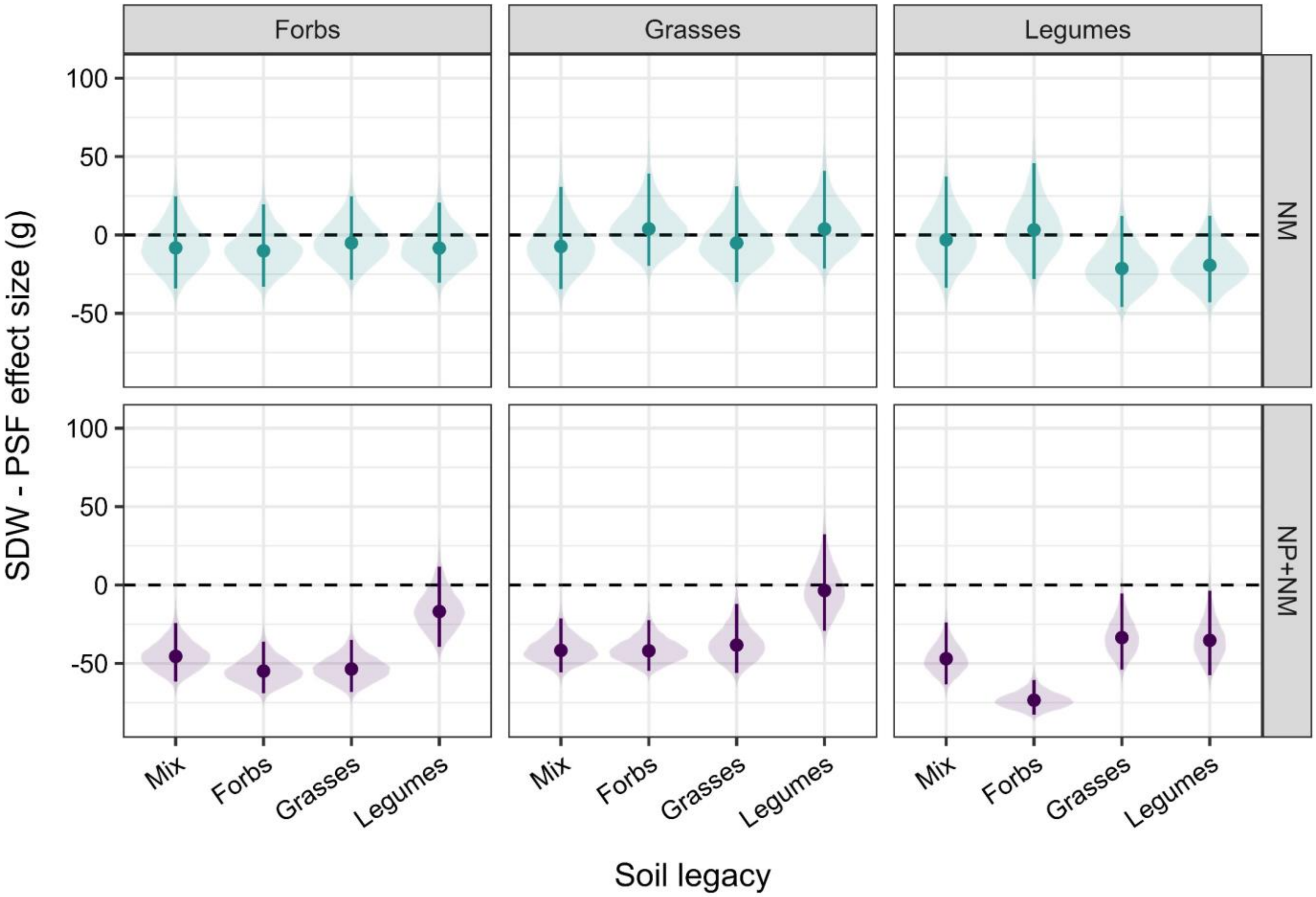


Figure S2. Relative effect size of shoot productivity (Shoot Dry Weight [g]) of forbs, grasses, legumes, and mixtures of PFG communities under soil niche modification (blue) or soil niche modification + soil niche preemption (purple) conditions. The dashed-line represent the zero value. Closed blue dots represent the average value of the effect sizes of the PFG growing under soil niche modification. Closed purple dots represent the average value of effect sizes of PFG growing under soil niche modification + soil niche preemption (purple) conditions.

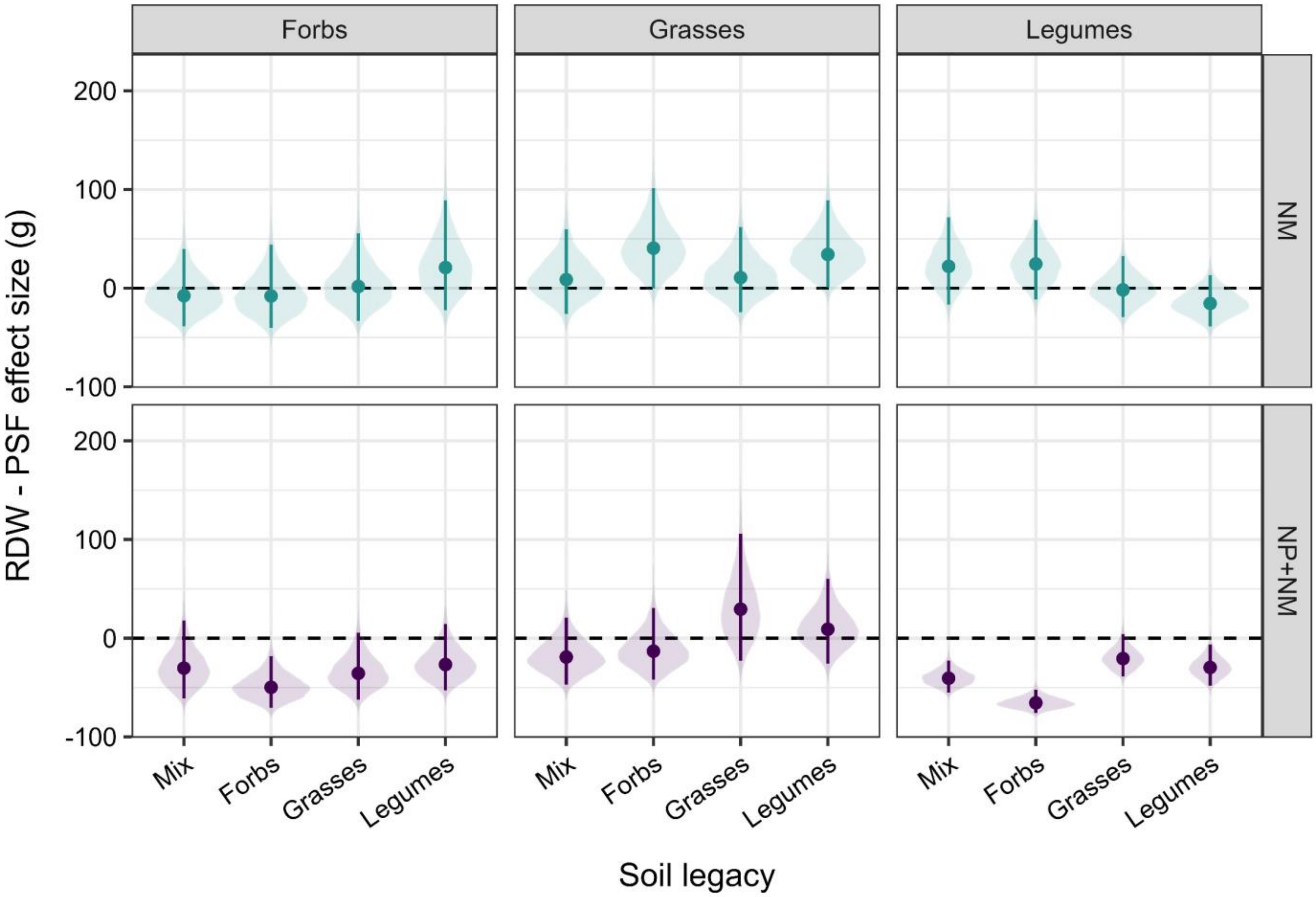


Figure S3. Relative effect size of shoot productivity (Root Dry Weight [g]) of forbs, grasses, legumes, and mixtures of PFG communities under soil niche modification (blue) or soil niche modification + soil niche preemption (purple) conditions. The dashed-line represent the zero value. Closed blue dots represent the average value of the effect sizes of the PFG growing under soil niche modification. Closed purple dots represent the average value of effect sizes of PFG growing under soil niche modification + soil niche preemption (purple) conditions.

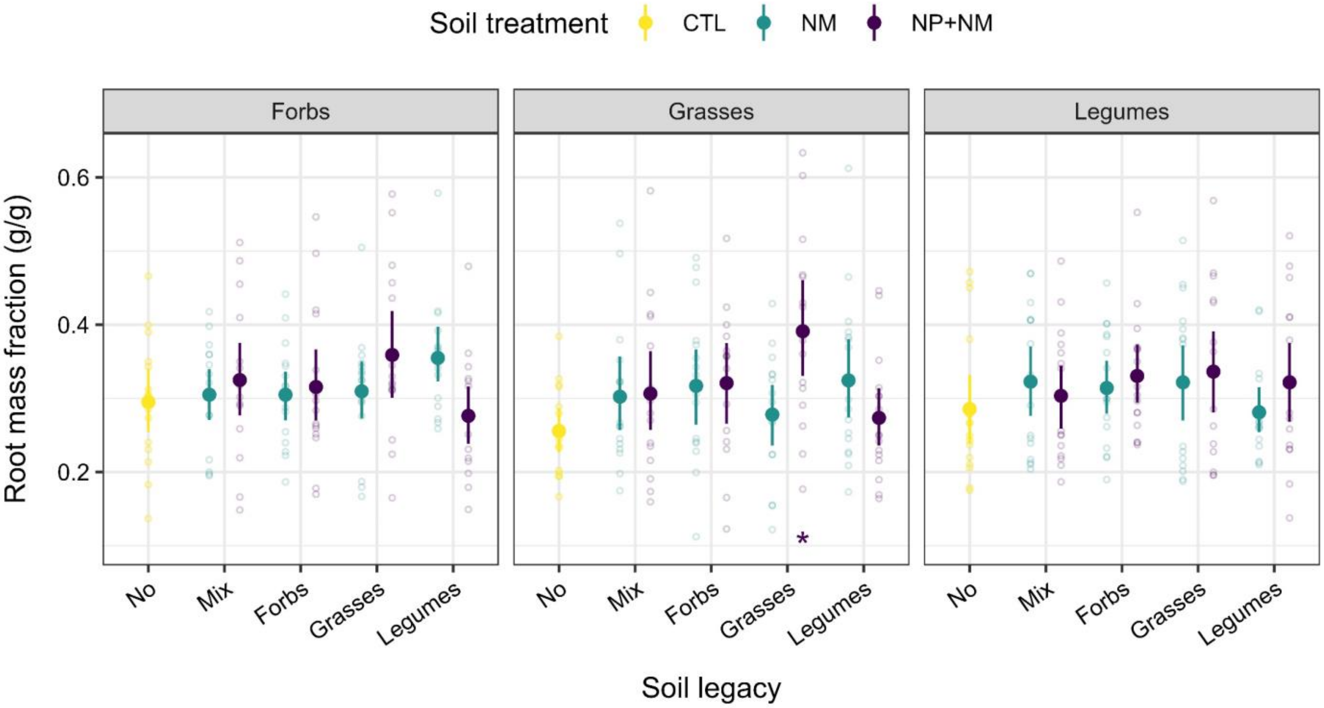


Figure S4. Root mass fraction (g/g) of forbs, grasses, and legumes under control (yellow), soil niche modification (blue), or soil niche modification + soil niche preemption (purple) soil conditions. Forbs, Grasses and Legumes on the top of the graph represent the PGF to which the plant species sown in the feedback phase belonged. Closed dots represent the mean value, and 95% confidence interval computed using non-parametric bootstrap are shown (n=45-48). Open dots represent the observed shoot or root dry weight values, which were jittered horizontally to improve the readability. On the x-axis, the soil legacies created in the conditioned soil are represented as follows: No: No legacy (this is our control), Mix: mix of PFG legacy, Forbs: forbs species legacy, Grasses: grasses species legacy, Legumes, legume species legacy. Groups marked with an asterisk are significantly different from the no legacy control (yellow group).

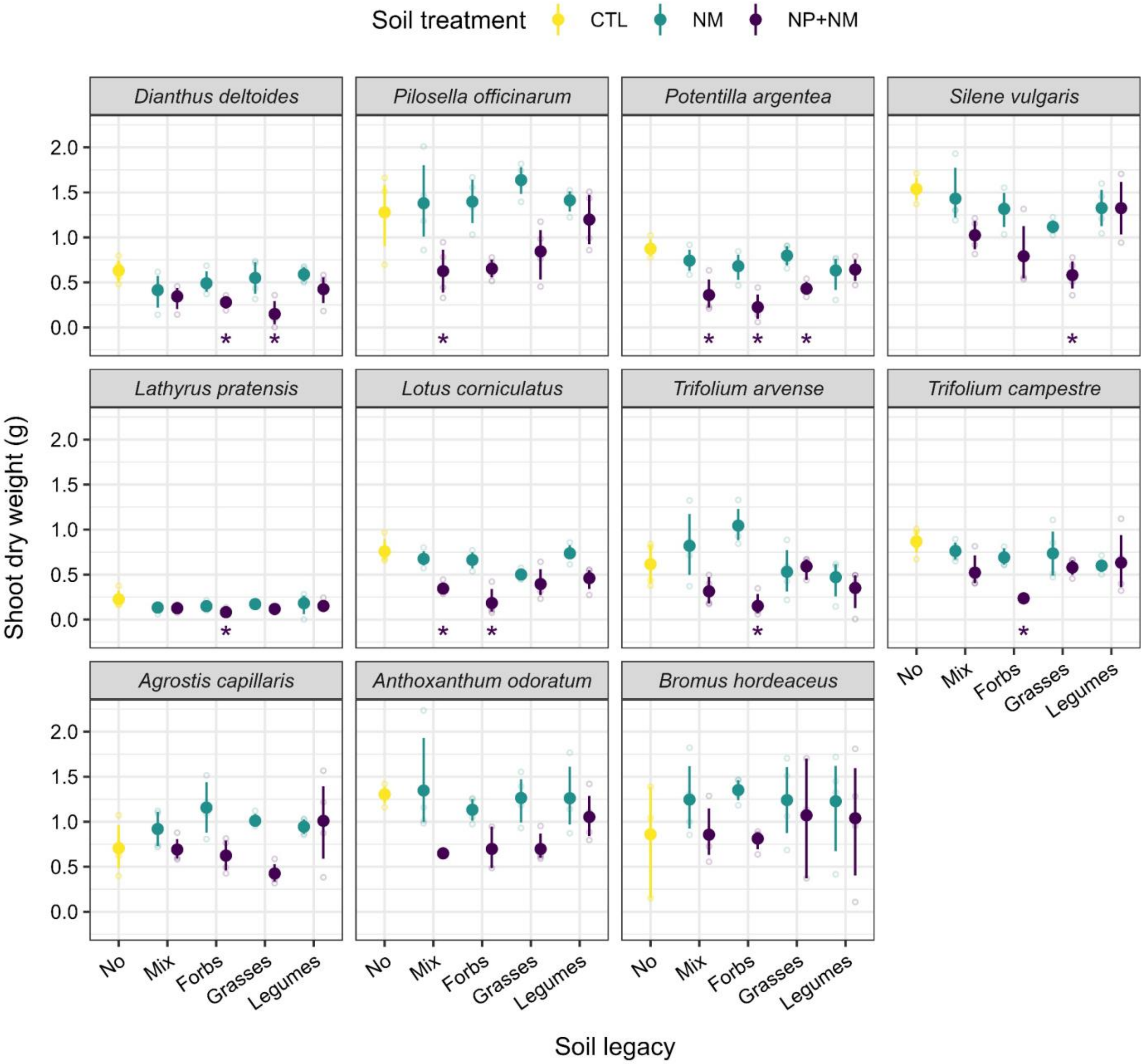


Figure S5. Shoot dry weight (g) of individual plant species under control (yellow), soil niche modification (blue) or soil niche modification + soil niche preemption (purple) soil conditions. Species names on the top of the graph represent the species identity of the plant sown in the feedback phase belonged. Closed dots represent the mean value, and 95% confidence interval computed using non-parametric bootstrap are shown (n=45-48). Open dots represent the observed shoot or root dry weight values, which were jittered horizontally to improve the readability. On the x-axis, the soil legacies created in the conditioned soil are represented as follows: No: No-legacy - control group, Mix: mix of PFG legacy, Forbs: forbs species legacy, Grasses: grasses species legacy, Legumes, legume species legacy. Groups marked with an asterisk are significantly different from the no legacy control (yellow group).

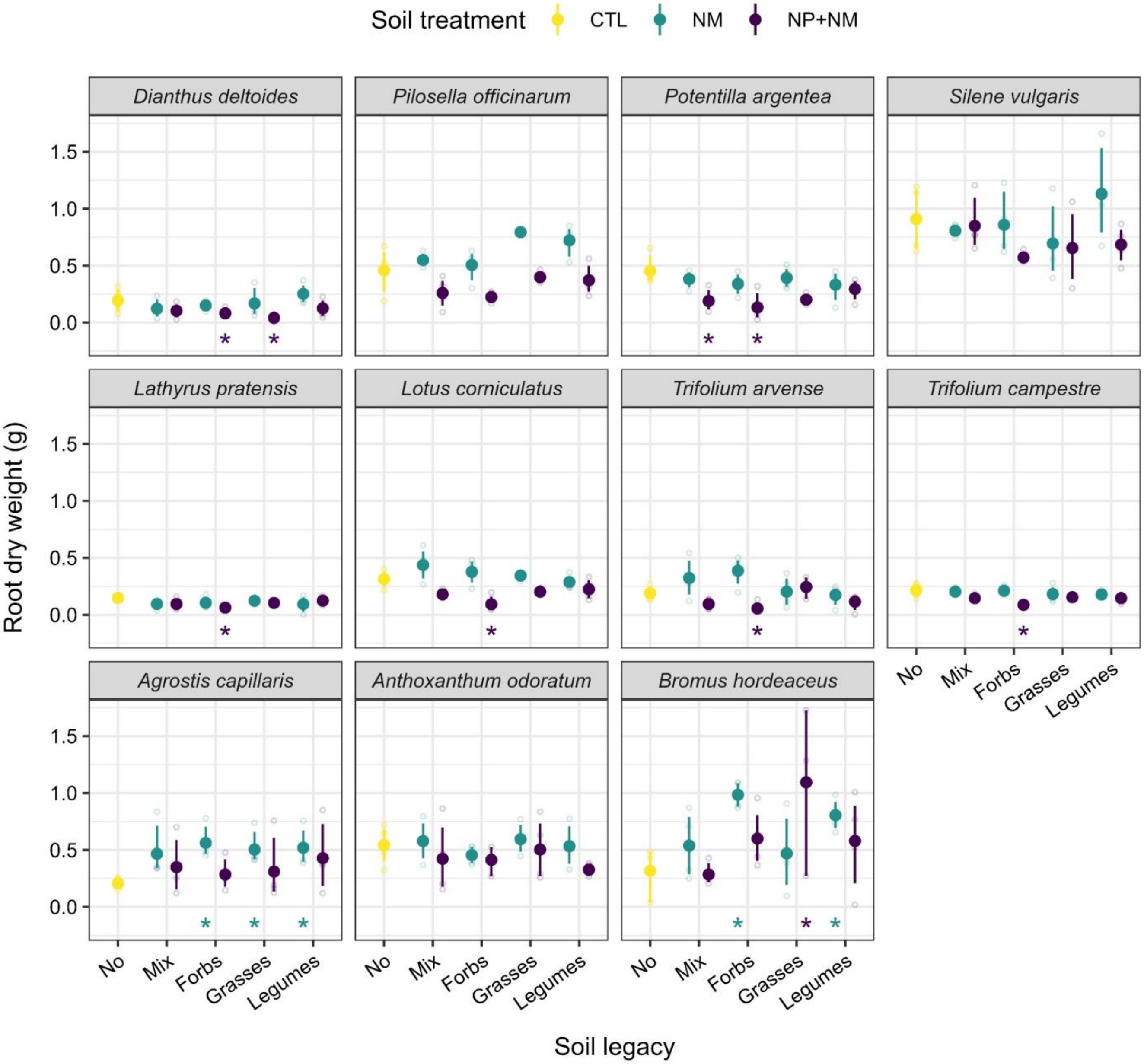
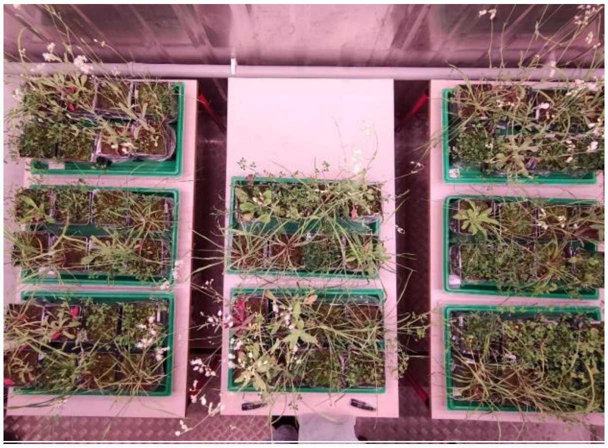


Figure S6. Root dry weight (g) of individual plant species under control (yellow), soil niche modification (blue) or soil niche modification + soil niche preemption (purple) soil conditions. Species names on the top of the graph represent the species identity of the plant sown in the feedback phase belonged. Closed dots represent the mean value, and 95% confidence interval computed using non-parametric bootstrap are shown (n=45-48). Open dots represent the observed shoot or root dry weight values, which were jittered horizontally to improve the readability. On the x-axis, the soil legacies created in the conditioned soil are represented as follows: No: No-legacy - control group, Mix: mix of PFG legacy, Forbs: forbs species legacy, Grasses: grasses species legacy, Legumes, legume species legacy. Groups marked with an asterisk are significantly different from the no legacy control (yellow group).

4.2 Supplementary photographic material of Chapter 3

FEBRUARY 2021
Nutrient flush pre-experiment



MARCH 2021
Nutrient flush pre-experiment

MARCH 2021
Nutrient flush pre-experiment

SEPTEMBER 2022
Conditioning phase pots –
Plant-soil feedback experiment

NOVEMBER 2022
Feedback phase pots –
Plant-soil feedback experiment

NOVEMBER 2022
Legume clean root

Synthesis and Conclusions

5. Synthesis and Conclusions

5.1 Main findings

In general, the findings presented in this thesis provide support for the importance of *priority* and *year effects* in terms of ecosystem structure and functioning in grasslands. These findings are relevant to the context of management and restoration of these ecosystems that have been identified as an important ecosystem that require attention during the next decade (Dudley et al., 2020; *UN Decade on Restoration*).

This thesis provides evidence that identifies the following findings:

- The order of arrival of plant functional groups did not affect the plant community productivity above or belowground ***(Chapter 1 and 2)***. However, aboveground productivity was strongly affected by the year of initiation of the plant communities. In contrast, species composition was driven by the time since establishment, with little contribution from the plant functional group order of arrival and year of initiation. Moreover, species diversity was modulated by the PFG order of arrival; however, this effect evolved over time and depended on the year of initiation of plant communities. ***(Chapter 1)***

- Assembly history can modulate the vertical root distribution of plants in grassland ecosystems. Plant functional group order of arrival affected vertical root distribution, with communities where grasses were the initial founders exhibiting shallower rooting, whereas deeper root systems were displayed in those in which forbs or legumes arrived first. ***(Chapters 1 and 2)***

- *Soil niche preemption* in combination with *soil niche modification* are responsible for the creation of *priority effects* in dry acidic grasslands, where *soil niche preemption* seems to be the primary driver in the creation of priority effects because *soil niche modification* alone did not produce significant effects on PFG development. ***(Chapter 3)***

5.2 Priority effects on community structure and productivity

Prior experimental studies that manipulated the order of arrival of plant functional groups in plant grassland communities observed that sowing legumes as the founder PFG resulted in communities with higher aboveground productivity, but lower belowground productivity (Körner et al., 2008; von Gillhaussen et al., 2014; Weidlich et al., 2017, 2018). However, the results presented in this thesis did not find a significant impact of different PFG arrival orders on above-or belowground productivity in the *POEM* or *Rhizobox* experiments. Instead, the year of initiation of the plant community had a significant effect on aboveground productivity in the *POEM* experiment. Consistent with these findings, previous experiments in Californian grasslands communities indicated that *year effects* was the strongest driver of changes in plant communities, although *priority effects* were also important in these cases to a lesser extend (Stuble et al., 2017.b; Werner et al., 2020) (Figure 5.1 and Figure 5.2).

Additionally, in the *POEM* experiment, the community composition was influenced by the time since the experiment was initiated, with a relatively smaller contribution of the plant functional group order of arrival and the year of initiation. From the second growing season, forbs first and grasses first communities were dominated by forbs and grasses, respectively. In contrast, diversity was driven by PFG order of arrival over time, depending on the year of initiation of the plant communities. In the third growing season, forbs first communities were the most diverse and grasses-first the least diverse. Previous studies have also reported *priority effects* on community composition, with communities dominated by the founder PFG (Körner et al., 2008; von Gillhaussen et al., 2014; Weidlich et al., 2017). In Werner et al., (2016) as in the results obtained in this thesis, *priority effects* increased over time with forbs and grasses dominating the communities where they arrived first. Although the *priority effects* results of this thesis were not very strong and acted in combination with time and year of initiation, there was still an impact of the order of arrival on the community structure (Figure 5.1).

Differences in experimental setup have an important impact on the strength of the obtained results in the structure and productivity of the communities by manipulating the order of arrival of plant functional groups. Here, it is argued that 1) the ecosystem and the experimental conditions could be a contributing factor to the variation observed in *priority effects* outcomes. In the *POEM* experiment, the selected system was a dry acidic grassland with nutrient-poor soil under field conditions, where no *priority effects* were identified in either aboveground or belowground productivity. In contrast, a previous field experiment conducted in a mesotrophic grassland with richer nutrient soil detected *priority effects* on productivity both above- and belowground (Weidlich et al., 2017, 2018). This is aligned with the ecological theory, which suggests that in environments with higher nutrient levels, competition tends to intensify (Chase, 2003; Chase & Leibold, 2009). In the *Rhizobox* experiment, the order of arrival of PFG was tested in a greenhouse experiment using rhizoboxes. The limited growth space provided by the rhizoboxes in combination with the short duration of the experiment, around 40 days, could have been the cause of the absence of *priority effects* in above- and belowground productivity. 2) The species composition of the PFG, with the inclusion of species with highly competitive characteristics in seed mixtures, could result in communities predominantly dominated by these species. In the *POEM* experiment, the composition of the communities evolved differently over time depending on the year of initiation and order of arrival of the PFG. However, the early arrival of grasses had a detrimental effect on plant diversity and evenness, primarily because of the strong dominance of *Bromus hordeaceus*. 3) The duration of the sowing interval has also been found to affect the strength of the priority effects (Körner et al., 2008; von Gillhaussen et al., 2014; Werner et al., 2020). In the *POEM* experiment, the sowing interval was six weeks, which might not have been long enough to create *priority effects* in a slow system such as dry acidic grasslands. 4) Management practices, such as mowing, have the potential to benefit perennial species (Kirmer et al., 2018), and weeding reduces competition for the sown species, thereby reinforcing priority effects, particularly when the sown species are of a perennial nature or less competitive than unsown species. The *POEM* experiment was only mowed once at the end of the season, and the treatment plots were never weeded, while other experiments that detected *priority effects* mowed twice a year (Weidlich et al., 2017) or weeded the plots from unwanted species (Stuble et al., 2017.b).

The next step in restoration ecology research would be to elucidate the extent to which different factors, such as site, soil characteristics, or the year of initiation, modulate the strength and direction of priority effects in determined ecosystems and conditions. Therefore, further field experiments are required to comprehensively assess the impact of specific factors on the outcomes of priority effects. The PFG approach, which involves manipulating the order of arrival of groups of species sharing common traits, provides a valuable framework for exploring how functional or morphological traits or phylogenetic relatedness respond to different environmental conditions. Furthermore, *year effects* have been demonstrated to be an important driver of aboveground productivity. It is important to determine the specific weather conditions that promote certain characteristics within communities. Additionally, research exploring whether this interaction between aboveground productivity and *year effects* also occurs at the belowground level is essential. This knowledge is vital for practical applications, particularly in ecological restoration. Considering the weather conditions that favour the desired species and encourage their development in the intended direction could enhance the success of restoration practices and achieve more effective ecosystem management.

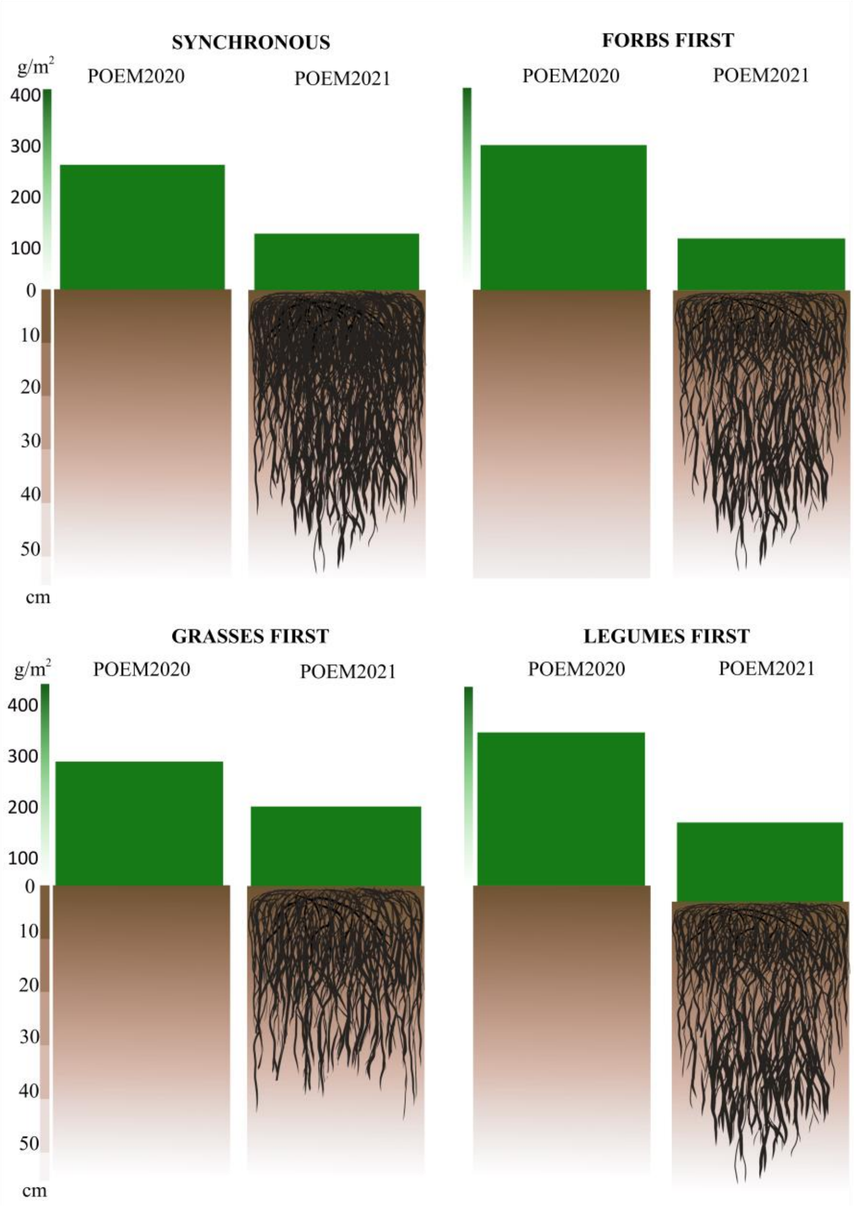


Figure 5.1. Synthesis of the results from the *POEM* experiment. Graphical representation of the *POEM* experiment communities of POEM2020 and POEM2021 in year three. Solid green bars represent the total biomass (g/m^2) of the communities of initiated in 2020 and the ones initiated in 2021. Roots represent differences in vertical distribution along the soil profile, depending on the order of arrival in the POEM2021 communities.

5.3 Priority effects on the vertical root distribution

This thesis sheds light on the fact that by manipulating the order of arrival of plant functional groups, *priority effects* can arise, boosting deeper root communities when forbs or legumes are the founders of PFG. The experiments here contained are the first exploring and detecting priority effects on the vertical root distribution of grassland plant communities. Similar results were obtained from both the *POEM* and *Rhizobox* experiments, despite the different experimental conditions tested. On the one hand, in the *POEM* experiment (Figure 5.1), the vertical root distribution generated by manipulating the order of arrival of PFG in dry acidic grasslands with species belonging to this ecosystem was tested under field conditions with a minirhizotron image system. On the other hand, in the *Rhizobox* experiment (Figure 5.2) this was tested under controlled conditions, with mesic grassland species, potting soil and a rhizobox imaging system.

The results indicated that by sowing grasses first, rooting patterns were shallower than sowing forbs or legumes, which generated deeper root systems without increasing root productivity. Different traits and adaptability of plants to abiotic and biotic conditions (Herben et al., 2018; Chen et al., 2020; Bakker et al., 2021, 2019; Lepik et al., 2021) created differences in vertical root distribution when a PFG arrived first. When species with deep-rooting patterns, such as forbs and legumes, arrive earlier, they can reach deeper soil layers and resources. They leave space for shallow rooting species, such as grasses, to grow in the topsoil. When species with shallow-rooting patterns arrive earlier (i.e., grasses), they preempt and modify the niche mainly in the topsoil, which can affect the colonization of deeper soil layers by deep-rooting species. Previous *priority effects* studies indicating differences in root productivity on the topsoil when sowing legumes first (Weidlich et al., 2018) could have underestimated root productivity in deeper layers of the soil by sampling only in shallow layers without considering the vertical distribution of the roots.

Introducing forbs or legumes first in grassland ecosystems can lead to deeper root communities, which could have potential implications for carbon storage in the subsoil because grasslands have been highlighted as a global carbon sink (Bai & Cotrufo, 2022; Dass et al., 2018). Deeper root systems could enhance ecosystem resilience, allowing plants to exploit resources and water at various levels (Fort et al., 2017; Maeght et al., 2013). This could contribute to increase niche differentiation (Leibold, 2008) by using the environment differently, thereby reducing interspecific competition and promoting coexistence. Further investigation at the species level is needed to identify the species that explore the soil vertically under these arrival circumstances using molecular techniques relying on DNA sequencing (Wagemaker et al., 2020).

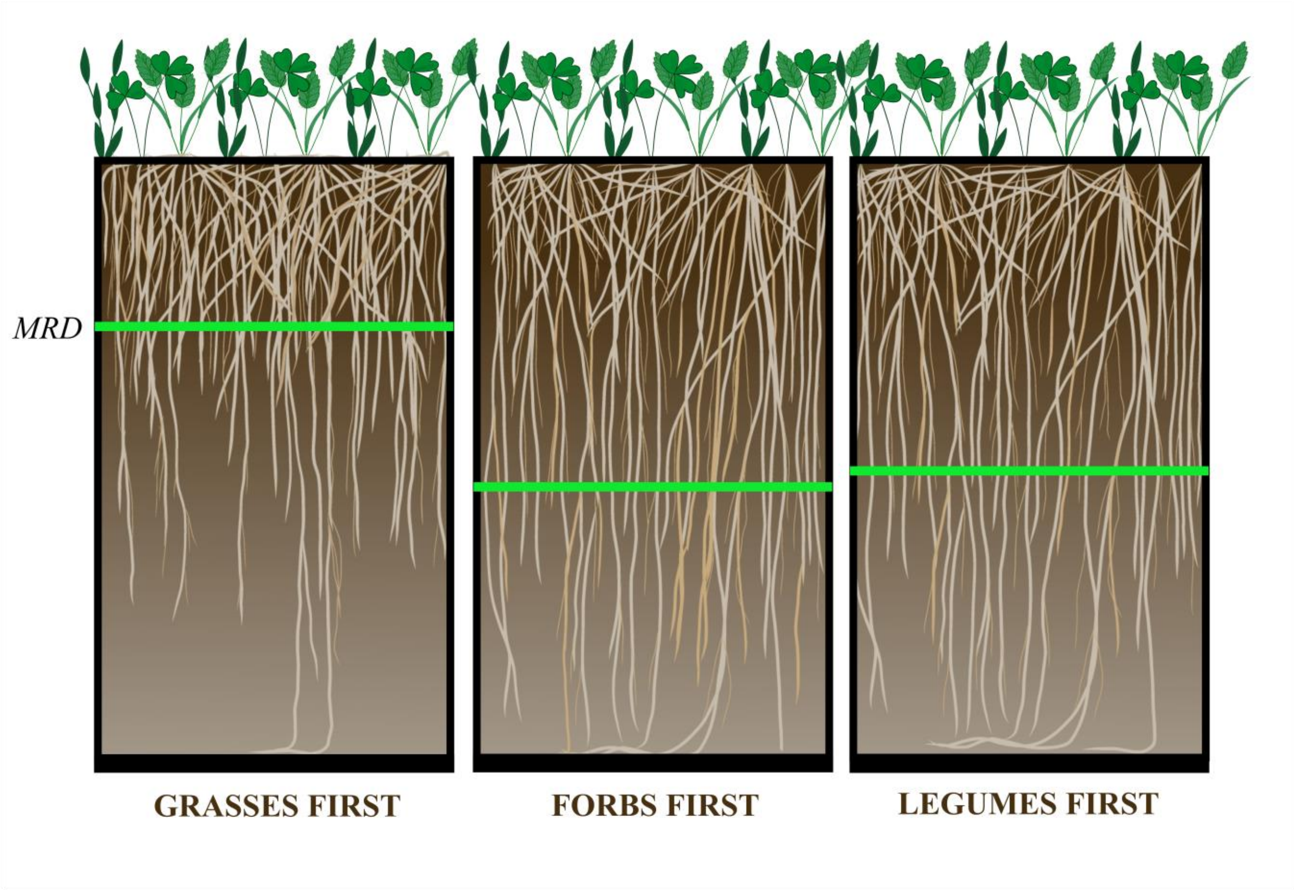


Figure 5.2. Synthesis of results from the *Rhizobox* experiment. Representation of the mean root depth (*MRD*) of grasses first, forbs first, and legumes first communities at the end of the experiment.

5.4 Priority effects mechanisms

Legacy effect is defined following (Cuddington, 2011) as "*impacts of a species on abiotic or biotic features of ecosystems that persist for a long time after the species has been extirpated or ceased activity and which have an effect on other species*". According to the niche framework presented by Fukami, (2015), *priority effects* are created by two classes of mechanisms: *niche preemption*, where species arriving earlier use the resources available in the niche (e.g., nutrients, light, or space), limiting them for the later arriving species, and *niche modification*, where species arriving earlier change the types of niches available for later arriving species (e.g., microbial communities or chemical exudates). The composition of grassland communities can be shaped by the effect of the mechanisms of *niche preemption* and *niche modification*, since once a species is established, its legacy could have long-lasting permanence in the system, thus affecting community assembly (Helsen et al., 2016). In the *PSF* experiment*,* the two mechanisms were isolated to focus solely on aspects related to the soil in a *plant-soil feedback* setup. *Soil niche preemption* (nutrient uptake) and *soil niche modification* (microbial legacies) were investigated to assess their significance in creating *priority effects* in dry acidic grassland plant communities.

The findings from this thesis show that *soil niche modification* did not create priority effects in the PFG. In contrast, when both mechanisms were applied together, *soil niche modification* and *soil niche preemption*, their combination affected PFG development, always with a negative response (Figure 5.3). Soil conditioned by mixtures of PFG, forbs, or grasses affected the aboveground development of all subsequent PFG in the feedback phase. Only legumes were affected by their own legacies. Forbs and mixture of PFG conditioned soils affected the belowground productivity of legumes and forbs, with forbs also affected by grass-conditioned soil. Grasses allocate more biomass to their roots, increasing their root mass fraction when they grew in their own conditioned soil, which could indicate higher intra- and/or interspecific competition between grasses species. The soil used in this experiment was a nutrient-poor sandy soil, which could be the key to these findings. Previous studies have suggested that abiotic soil conditions trigger *plant-soil feedback* responses rather than microbial legacies in sandy soils (Bezemer et al., 2006). Nutrient depletion via *soil niche preemption* seemed to be the primary driver in the creation of *priority effects*, as *soil niche modification* alone did not significantly impact PFG development. The uptake of specific macro and/or micronutrients by early arrival PFG could have created a more restricted environment for the development of later arrival PFG than that created by microbial legacies.

These results indicate that in slow systems, such as dry acidic grasslands with nutrient-poor soils, *soil niche preemption* through nutrient depletion by early arrival PFG has an important impact on subsequent PFG arrivals. These findings are context-dependent because, in nature, these processes are never isolated; instead, they interact with other mechanisms such as competition for light, water, or space. However, these additional factors were not considered in the present study. Therefore, further investigation at the microbiome level is needed to clarify the absence of a significant response to soil niche modification.

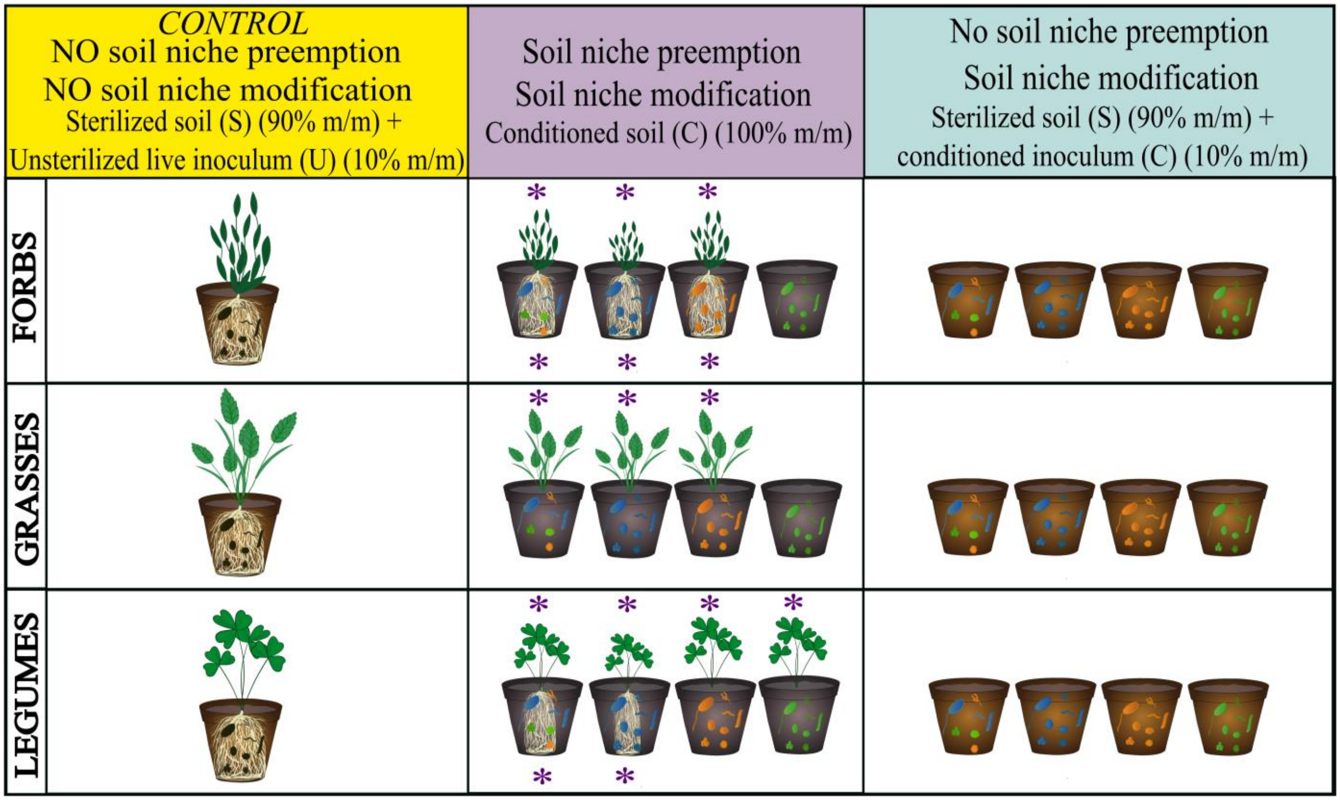


Figure 5.3. Synthesis of the results presented in *Chapter 2*. Representation of the significant differences in shoot and root growth of the plant functional groups, forbs, grasses, and legumes in response (feedback phase) to the soil treatments established with the legacies created by mix, forbs, grasses, and legumes communities during the conditioning phase. * represents significant differences compared with the control. The absence of shoots and roots in the representation indicates that no significant differences.

5.5 Conclusions

In conclusion, the findings of this thesis underscore the significance of manipulating the order of arrival of PFG in creating priority effects within the vertical root distribution of grassland systems. Specifically, sowing legumes and forbs ahead of other PFG results in deeper root communities, providing a method that could enhance resilience in grassland ecosystems against harsh weather conditions, promote niche differentiation, and consequently foster species coexistence. The *POEM* and *Rhizobox* experiments are the first to test and detect priority effects in vertical root distribution.

At the same time, *year effects*, considered as the weather conditions that the communities endured during their year of initiation, were found to be a significant factor influencing aboveground productivity in dry acidic grasslands. Therefore, identifying the specific weather conditions that support the preferred trajectory of community assembly could represent a crucial advancement in the field of restoration. Moreover, it is important to investigate the extent to which year effects also affect below-ground productivity.

Furthermore, the findings from the *PSF* experiment suggest that the interplay between soil niche preemption and soil niche modification is key to understanding the priority effects responses in dry acidic grasslands. From available evidence, it is plausible that nutrient uptake may be the primary factor driving these responses in such ecosystems.

Collectively, these results contribute to a better understanding of community assembly, providing insights that could prove beneficial in designing restoration strategies aimed at fostering specific ecosystem functions in grasslands.

6. References

References of 1. Thesis framework and 5. Synthesis and Conclusions.

Atkinson, J., Groves, A. M., Towers, I. R., Catano, C. P., & Brudvig, L. A. (2023). Trait-mediated community assembly during experimental grassland restoration is altered by planting year rainfall. *The Journal of Applied Ecology*, *60*(8), 1587–1596.

Bai, Y., & Cotrufo, M. F. (2022). Grassland soil carbon sequestration: Current understanding, challenges, and solutions. *Science*, *377*(6606), 603–608.

Bakker, J. D., Wilson, S. D., Christian, J. M., Li, X., Ambrose, L. G., & Waddington, J. (2003). Contingency of grassland restoration on year, site, and competition from introduced grasses. *Ecological Applications: A Publication of the Ecological Society of America*, *13*(1), 137–153.

Bakker, L. M., Barry, K. E., Mommer, L., & van Ruijven, J. (2021). Focusing on individual plants to understand community scale biodiversity effects: the case of root distribution in grasslands. *Oikos* , *130*(11), 1954–1966.

Bakker, L. M., Mommer, L., & van Ruijven, J. (2019). Using root traits to understand temporal changes in biodiversity effects in grassland mixtures. *Oikos* , *128*(2), 208–220.

Bengtsson, J., Bullock, J. M., Egoh, B., Everson, C., Everson, T., O'Connor, T., O'Farrell, P. J., Smith, H. G., & Lindborg, R. (2019). Grasslands-more important for ecosystem services than you might think. *Ecosphere* , *10*(2), e02582.

Bengtsson, J., Fagerström, T., & Rydin, H. (1994). Competition and coexistence in plant communities. *Trends in Ecology & Evolution*, *9*(7), 246–250.

Bennett, J. A., & Klironomos, J. (2019). Mechanisms of plant-soil feedback: interactions among biotic and abiotic drivers. *The New Phytologist*, *222*(1), 91–96.

Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology & Evolution*, *9*(5), 191–193.

Bezemer, T. M., Lawson, C. S., Hedlund, K., Edwards, A. R., Brook, A. J., Igual, J. M., Mortimer, S. R., & Van Der Putten, W. H. (2006). Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *The Journal of Ecology*, *94*(5), 893–904.

Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J. M. J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C. L., … Michalet, R. (2008). Facilitation in Plant Communities: The Past, the Present, and the Future. *The Journal of Ecology*, *96*(1), 18–34.

Buchmann, T., Schumacher, J., Ebeling, A., Eisenhauer, N., Fischer, M., Gleixner, G., Hacker, N., Lange, M., Oelmann, Y., Schulze, E.-D., Weigelt, A., Weisser, W. W., Wilcke, W., & Roscher, C. (2018). Connecting experimental biodiversity research to real-world grasslands. *Perspectives in Plant Ecology, Evolution and Systematics*, *33*, 78–88.

Buisson, E., Le Stradic, S., Silveira, F. A. O., Durigan, G., Overbeck, G. E., Fidelis, A., Fernandes, G. W., Bond, W. J., Hermann, J.-M., Mahy, G., Alvarado, S. T., Zaloumis, N. P., & Veldman, J. W. (2019). Resilience and restoration of tropical and subtropical grasslands, savannas, and grassy woodlands. *Biological Reviews of the Cambridge Philosophical Society*, *94*(2), 590–609.

Callaway, R. M. (1998). Are Positive Interactions Species-Specific? *Oikos* , *82*(1), 202–207.

Catano, C. P., Groves, A. M., & Brudvig, L. A. (2023). Community assembly history alters relationships between biodiversity and ecosystem functions during restoration. *Ecology*, *104*(2), e3910.

Cesarano, G., Zotti, M., Antignani, V., Marra, R., Scala, F., & Bonanomi, G. (2017). SOIL SICKNESS AND NEGATIVE PLANT-SOIL FEEDBACK: A REAPPRAISAL OF HYPOTHESES. *Journal of Plant Pathology: An International Journal of the Italian Phytopathological Society*, *99*(3), 545–570.

Chase, J. M. (2003). Community assembly: when should history matter? *Oecologia*, *136*(4), 489–498.

Chase, J. M., & Leibold, M. A. (2009). *Ecological Niches: Linking Classical and Contemporary Approaches*. University of Chicago Press.

Chen, B. J. W., Xu, C., Liu, M. S., Huang, Z. Y. X., Zhang, M. J., Tang, J., & Anten, N. P. R. (2020). Neighbourhood-dependent root distributions and the consequences on root separation in arid ecosystems. *The Journal of Ecology*, *108*(4), 1635–1648.

Clements, F. E. (1936). Nature and Structure of the Climax. *The Journal of Ecology*, *24*(1), 252–284.

Collinge, S. K., & Ray, C. (2009). Transient patterns in the assembly of vernal pool plant communities. *Ecology*, *90*(12), 3313–3323.

Cowles, H. C. (1899). The Ecological Relations of the Vegetation on the Sand Dunes of Lake Michigan. Part I.-Geographical Relations of the Dune Floras. *Botanical Gazette* , *27*(2), 95–117.

Crawley, M. J. (2004). Timing of disturbance and coexistence in a species-rich ruderal plant community. *Ecology*, *85*(12), 3277–3288.

Cuddington, K. (2011). Legacy Effects: The Persistent Impact of Ecological Interactions. *Biological Theory*, *6*(3), 203–210.

Dass, P., Houlton, B. Z., Wang, Y., & Warlind, D. (2018). Grasslands may be more reliable carbon sinks than forests in California. *Environmental Research Letters*, *13*(7), 074027.

del Moral, R., Sandler, J. E., & Muerdter, C. P. (2009). Spatial factors affecting primary succession on the Muddy River Lahar, Mount St. Helens, Washington. *Plant Ecology*, *202*(1), 177–190.

Delory, B. M., Schempp, H., Spachmann, S. M., Störzer, L., van Dam, N. M., Temperton, V. M., & Weinhold, A. (2021). Soil chemical legacies trigger species-specific and context-dependent root responses in later arriving plants. *Plant, Cell & Environment*, ce.13999.

Delory, B. M., Weidlich, E. W. A., von Gillhaussen, P., & Temperton, V. M. (2019). When history matters: The overlooked role of priority effects in grassland overyielding. *Functional Ecology*, *33*(12), 2369–2380.

Diamond, J. M. (1975). Assembly of species communities. *Ecology and Evolution of Communities*, 342–444.

Dudley, N., Eufemia, L., Fleckenstein, M., Periago, M. E., Petersen, I., & Timmers, J. F. (2020). Grasslands and savannahs in the UN Decade on Ecosystem Restoration. *Restoration Ecology*, *28*(6), 1313–1317.

Ejrnaes, R., Bruun, H. H., & Graae, B. J. (2006). Community assembly in experimental grasslands: suitable environment or timely arrival? *Ecology*, *87*(5), 1225–1233.

Fort, F., Volaire, F., Guilioni, L., & Barkaoui, K. (2017). Root traits are related to plant water-use among rangeland Mediterranean species. *Functional Ecology, 31*(9), 1700-1709.

Fukami, T. (2015). Historical contingency in community assembly : integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics*, *46*, 1–23.

Fukami, T., & Nakajima, M. (2011). Community assembly: alternative stable states or alternative transient states? *Ecology Letters*, *14*(10), 973–984.

Gibson, D. J. (2009). *Grasses and Grassland Ecology*. Oxford University Press.

Gleason, H. A. (1926). The Individualistic Concept of the Plant Association. *Bulletin of the Torrey Botanical Club*, *53*(1), 7–26.

Goldberg, D. E. (1996). Competitive Ability: Definitions, Contingency and Correlated Traits. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *351*(1345), 1377–1385.

Goldberg, D. E., & Barton, A. M. (1992). Patterns and Consequences of Interspecific Competition in Natural Communities: A Review of Field Experiments with Plants. *The American Naturalist*, *139*(4), 771–801.

Grman, E., Bassett, T., & Brudvig, L. A. (2013). Confronting contingency in restoration: management and site history determine outcomes of assembling prairies, but site characteristics and landscape context have little effect. *The Journal of Applied Ecology*, *50*(5), 1234–1243.

Groves, A. M., & Brudvig, L. A. (2019). Interannual variation in precipitation and other planting conditions impacts seedling establishment in sown plant communities. *Restoration Ecology*, *27*(1), 128–137.

Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., & Wiezik, M. (2013). European grassland ecosystems: threatened hotspots of biodiversity. *Biodiversity and Conservation*, *22*(10), 2131–2138.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G., Finn, J. A., Freitas, H., Giller, P. S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Korner, C., Leadley, P. W., Loreau, M., Minns, A., … Lawton, J. H. (1999). Plant diversity and productivity experiments in european grasslands. *Science*, *286*(5442), 1123–1127.

Helsen, K., Hermy, M., & Honnay, O. (2012). Trait but not species convergence during plant community assembly in restored semi-natural grasslands. *Oikos* , *121*(12), 2121–2130.

Helsen, K., Hermy, M., & Honnay, O. (2016). A test of priority effect persistence in semi-natural grasslands through the removal of plant functional groups during community assembly. *BMC Ecology*, *16*, 22.

Herben, T., Vozábová, T., Hadincová, V., Krahulec, F., Mayerová, H., Pecháčková, S., Skálová, H., & Krak, K. (2018). Vertical root distribution of individual species in a mountain grassland community: Does it respond to neighbours? *The Journal of Ecology*, *106*(3), 1083–1095.

Hess, M. C. M., Buisson, E., Fontes, H., Bacon, L., Sabatier, F., & Mesléard, F. (2020). Giving recipient communities a greater head start and including productive species boosts early resistance to invasion. *Applied Vegetation Science*, *23*(3), 340–352.

Huston, M., & Smith, T. (1987). Plant Succession: Life History and Competition. *The American Naturalist*, *130*(2), 168–198.

Isbell, F. I., & Wilsey, B. J. (2011). Increasing native, but not exotic, biodiversity increases aboveground productivity in ungrazed and intensely grazed grasslands. *Oecologia*, *165*(3), 771–781.

Jesch, A., Barry, K. E., Ravenek, J. M., Bachmann, D., Strecker, T., Weigelt, A., Buchmann, N., de Kroon, H., Gessler, A., Mommer, L., Roscher, C., & Scherer-Lorenzen, M. (2018). Below-ground resource partitioning alone cannot explain the biodiversity-ecosystem function relationship: A field test using multiple tracers. *The Journal of Ecology*, *106*(5), 2002–2018.

Keddy, P. A., & Shipley, B. (1989). Competitive Hierarchies in Herbaceous Plant Communities. *Oikos* , *54*(2), 234–241.

Kelt, D. A., Taper, M. L., & Meserve, P. L. (1995). Assessing the impact of competition on community assembly: a case study using small mammals. *Ecology*, *76*(4), 1283-1296.

Kirmer, A., Rydgren, K., & Tischew, S. (2018). Smart management is key for successful diversification of field margins in highly productive farmland. *Agriculture, Ecosystems & Environment*, *251*, 88–98.

Körner, C., Stöcklin, J., Reuther-Thiébaud, L., & Pelaez-Riedl, S. (2008). Small differences in arrival time influence composition and productivity of plant communities. *The New Phytologist*, *177*(3), 698–705.

Lambers, J. H. R., Harpole, W. S., Tilman, D., Knops, J., & Reich, P. B. (2004). Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. *Ecology Letters*, *7*(8), 661–668.

Leibold, M. A. (2008, July 2). *Return of the niche*. *Nature*, *454*(7200), 39-41.

Lepik, A., Abakumova, M., Davison, J., Zobel, K., & Semchenko, M. (2021). Spatial mapping of root systems reveals diverse strategies of soil exploration and resource contest in grassland plants. *The Journal of Ecology*, *109*(2), 652–663.

Lewontin, R. C. (1969). The meaning of stability. *Brookhaven Symposia in Biology*, *22*, 13–24.

MacDougall, A. S., Wilson, S. D., & Bakker, J. D. (2008). Climatic variability alters the outcome of long-term community assembly. *The Journal of Ecology*, *96*(2), 346–354.

Maeght, J.-L., Rewald, B., & Pierret, A. (2013). How to study deep roots—and why it matters. *Frontiers in Plant Science*, *4,* 299.

Marquard, E., Weigelt, A., Temperton, V. M., Roscher, C., Schumacher, J., Buchmann, N., Fischer, M., Weisser, W. W., & Schmid, B. (2009). Plant species richness and functional composition drive overyielding in a six-year grassland experiment. *Ecology*, *90*(12), 3290–3302.

Martin, L. M., & Wilsey, B. J. (2012). Assembly history alters alpha and beta diversity, exotic-native proportions and functioning of restored prairie plant communities. *The Journal of Applied Ecology*, *49*(6), 1436–1445.

McMahen, K., Guichon, S. H. A., Anglin, C. D., Lavkulich, L. M., Grayston, S. J., & Simard, S. W. (2022). Soil microbial legacies influence plant survival and growth in mine reclamation. *Ecology and Evolution*, *12*(11), e9473.

Meyer, S. T., Ebeling, A., Eisenhauer, N., Hertzog, L., Hillebrand, H., Milcu, A., Pompe, S., Abbas, M., Bessler, H., Buchmann, N., De Luca, E., Engels, C., Fischer, M., Gleixner, G., Hudewenz, A., Klein, A.-M., Kroon, H., Leimer, S., Loranger, H., … Weisser, W. W. (2016). Effects of biodiversity strengthen over time as ecosystem functioning declines at low and increases at high biodiversity. *Ecosphere* , *7*(12), e01619.

Morton, R. D., & Law, R. (1997). Regional Species Pools and the Assembly of Local Ecological Communities. *Journal of Theoretical Biology*, *187*(3), 321–331.

Mueller, K. E., Tilman, D., Fornara, D. A., & Hobbie, S. E. (2013). Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. *Ecology*, *94*(4), 787–793.

Oelmann, Y., Buchmann, N., Gleixner, G., Habekost, M., Roscher, C., Rosenkranz, S., Schulze, E.-D., Steinbeiss, S., Temperton, V. M., Weigelt, A., Weisser, W. W., & Wilcke, W. (2011). Plant diversity effects on aboveground and belowground N pools in temperate grassland ecosystems: Development in the first 5 years after establishment. *Global Biogeochemical Cycles*, *25*(2).

Oram, N. J., Ravenek, J. M., Barry, K. E., Weigelt, A., Chen, H., Gessler, A., Gockele, A., de Kroon, H., van der Paauw, J. W., Scherer-Lorenzen, M., Smit-Tiekstra, A., van Ruijven, J., & Mommer, L. (2018). Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. *The Journal of Ecology*, *106*(1), 265–277.

Pärtel, M., Bruun, H. H., & Sammul, M. (2005). Biodiversity in temperate European grasslands: origin and conservation. *Grassland Science in Europe*, *10*(1), 14.

Pitt, M. D., & Heady, H. F. (1978). Responses of annual vegetation to temperature and rainfall patterns in northern California. *Ecology*, *59*(2), 336–350.

Poschlod, P., Baumann, A., & Karlik, P. (2009). Origin and development of grasslands in Central Europe. In *Grasslands in Europe* (pp. 15–25). KNNV Publishing.

Ravenek, J. M., Bessler, H., Engels, C., Scherer-Lorenzen, M., Gessler, A., Gockele, A., De Luca, E., Temperton, V. M., Ebeling, A., Roscher, C., Schmid, B., Weisser, W. W., Wirth, C., de Kroon, H., Weigelt, A., & Mommer, L. (2014). Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. *Oikos* , *123*(12), 1528–1536.

Rice, E. L. (2012). *Allelopathy*. Academic Press.

Roscher, C., Temperton, V. M., Scherer-Lorenzen, M., Schmitz, M., Schumacher, J., Schmid, B., Buchmann, N., Weisser, W. W., & Schulze, E.-D. (2005). Overyielding in experimental grassland communities - irrespective of species pool or spatial scale. *Ecology Letters*, *8*(4), 419–429.

Ruijven, J., De Deyn, G. B., & Berendse, F. (2003). Diversity reduces invasibility in experimental plant communities: the role of plant species. *Ecology Letters*, *6*(10), 910–918.

Sarneel, J. M., Kardol, P., & Nilsson, C. (2016). The importance of priority effects for riparian plant community dynamics. *Journal of Vegetation Science: Official Organ of the International Association for Vegetation Science*, *27*(4), 658–667.

Steinbeiss, S., Beßler, H., Engels, C., Temperton, V. M., Buchmann, N., Roscher, C., Kreutziger, Y., Baade, J., Habekost, M., & Gleixner, G. (2008). Plant diversity positively affects short-term soil carbon storage in experimental grasslands. *Global Change Biology*, *14*(12), 2937–2949.

Stevens, J. M., & Fehmi, J. S. (2011). Early establishment of a native grass reduces the competitive effect of a non-native grass. *Restoration Ecology*, *19*(3), 399–406.

Stuble, K. L., Fick, S. E., & Young, T. P. (2017.a). Every restoration is unique: testing year effects and site effects as drivers of initial restoration trajectories. *The Journal of Applied Ecology*, *54*(4), 1051–1057.

Stuble, K. L., & Souza, L. (2016). Priority effects: natives, but not exotics, pay to arrive late. *The Journal of Ecology*, *104*(4), 987–993.

Stuble, K. L., & Young, T. P. (2020). Priority Treatment Leaves Grassland Restoration Vulnerable to Invasion. *Diversity*, *12*(2), 71.

Stuble, K. L., Zefferman, E. P., Wolf, K. M., Vaughn, K. J., & Young, T. P. (2017.b). Outside the envelope: rare events disrupt the relationshipbetween climate factors and species interactions. *Ecology*, *98*(6), 1623–1630.

Sutherland, J. P. (1974). Multiple stable points in natural communities. *The American Naturalist*, *108*(964), 859–873.

Tansley, A. G. (1917). On Competition Between Galium Saxatile L. (G. Hercynicum Weig.) and Galium Sylvestre Poll. (G. Asperum Schreb.) On Different Types of Soil. *The Journal of Ecology*, *5*(3/4), 173–179.

Temperton, V. M., Baasch, A., von Gillhaussen, P., & Kirmer, A. (2016). Assembly theory for restoring ecosystem structure and functioning: timing is everything?. *Foundations of restoration ecology*, 245-270.

Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B., & Buchmann, N. (2007). Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia*, *151*(2), 190–205.

Tilman, D. (1982). Resource competition and community structure. *Monographs in Population Biology*, *17*, 1–296.

Tilman, David, Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The Influence of Functional Diversity and Composition on Ecosystem Processes. *Science*, *277*(5330), 1300–1302.

UN Decade on Restoration. UN Decade on Restoration. Retrieved November 16, 2023, from https://www.decadeonrestoration.org van der Plas, F. (2019). Biodiversity and ecosystem functioning in naturally assembled communities. *Biological Reviews of the Cambridge Philosophical Society*, *94*(4), 1220–1245.

Vaughni, K. J., & Young, T. P. (2015). Short-term priority over exotic annuals increases the initial density and longer-term cover of native perennial grasses. *Ecological Applications: A Publication of the Ecological Society of America*, *25*(3), 791–799.

von Gillhaussen, P., Rascher, U., Jablonowski, N. D., Plückers, C., Beierkuhnlein, C., & Temperton, V. M. (2014). Priority effects of time of arrival of plant functional groups override sowing interval or density effects: a grassland experiment. *PloS One*, *9*(1), e86906.

Wagemaker, C. A. M., Mommer, L., Visser, E. J. W., Weigelt, A., van Gurp, T. P., Postuma, M., Smit-Tiekstra, A. E., & de Kroon, H. (2020). msGBS: A new high-throughput approach to quantify the relative species abundance in root samples of multispecies plant communities. *Molecular Ecology Resources*, *21*(4), 1021–1036.

Wainwright, C. E., Wolkovich, E. M., & Cleland, E. E. (2012). Seasonal priority effects: implications for invasion and restoration in a semi-arid system. *The Journal of Applied Ecology*, *49*(1), 234–241.

Weidlich, E. W. A., von Gillhaussen, P., Delory, B. M., Blossfeld, S., Poorter, H., & Temperton, V. M. (2017). The Importance of Being First: Exploring Priority and Diversity Effects in a Grassland Field Experiment. *Frontiers in Plant Science*, *7*(2008), 1–12.

Weidlich, E. W. A., von Gillhaussen, P., Max, J. F. J., Delory, B. M., Jablonowski, N. D., Rascher, U., & Temperton, V. M. (2018). Priority effects caused by plant order of arrival affect below-ground productivity. *The Journal of Ecology*, *106*(2), 774–780.

Weisser, W. W., Roscher, C., Meyer, S. T., Ebeling, A., Luo, G., Allan, E., Beßler, H., Barnard, R. L., Buchmann, N., Buscot, F., Engels, C., Fischer, C., Fischer, M., Gessler, A., Gleixner, G., Halle, S., Hildebrandt, A., Hillebrand, H., de Kroon, H., … Eisenhauer, N. (2017). Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic and Applied Ecology*, *23*, 1–73.

Werner, C. M., Stuble, K. L., Groves, A. M., & Young, T. P. (2020). Year effects: Interannual variation as a driver of community assembly dynamics. *Ecology*, *101*(9), e03104.

Werner, C. M., Vaughn, K. J., Stuble, K. L., Wolf, K., & Young, T. P. (2016). Persistent asymmetrical priority effects in a California grassland restoration experiment. *Ecological Applications: A Publication of the Ecological Society of America*, *26*(6), 1624–1632.

Werner, C. M., Young, D. J. N., Safford, H. D., & Young, T. P. (2019). Decreased snowpack and warmer temperatures reduce the negative effects of interspecific competitors on regenerating conifers. *Oecologia*, *191*(4), 731–743.

White, R. P., Murray, S., Rohweder, M., Prince, S. D., & Thompson, K. M. (2000). *Grassland ecosystems* (p. 81). Washington, DC, USA: World Resources Institute.

Wilsey, B. (2020). Restoration in the face of changing climate: importance of persistence, priority effects, and species diversity. *Restoration Ecology*, *rec.13132*.

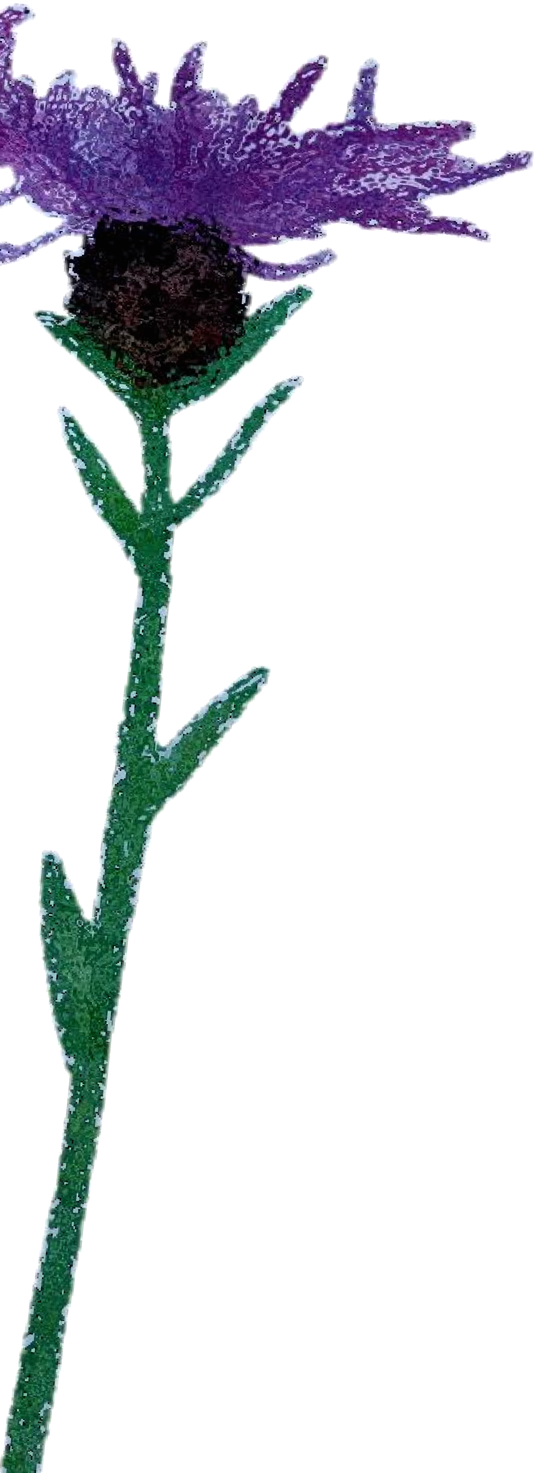
Wilsey, B. J., Barber, K., & Martin, L. M. (2015). Exotic grassland species have stronger priority effects than natives regardless of whether they are cultivated or wild genotypes. *The New Phytologist*, *205*(2), 928–937.

Wilson, J. B., Peet, R. K., Dengler, J., & Pärtel, M. (2012). Plant species richness: the world records. *Journal of Vegetation Science: Official Organ of the International Association for Vegetation Science*, *23*(4), 796–802.

Wright, A. J., Wardle, D. A., Callaway, R., & Gaxiola, A. (2017). The Overlooked Role of Facilitation in Biodiversity Experiments. *Trends in Ecology & Evolution*, *32*(5), 383–390.

Young, T. P., Zefferman, E. P., Vaughn, K. J., & Fick, S. (2015). Initial success of native grasses is contingent on multiple interactions among exotic grass competition, temporal priority, rainfall and site effects. *AoB Plants*, *7,* plus081.

Appendices



List of appendices

- List of figures
- Anlage 4 - Gazette 72/23 DE
- Appendix 4 Gazette 72/23 EN
- Authorship declaration
- Curriculum Vitae

List of figures

Anlage 4

Zu § 9 Eröffnung des Promotionsverfahrens

Muster Erklärungen und Versicherung

Inés María Alonso-Crespo

ines.alonso_crespo@leuphana.de / inesalonsocrespo@gmail.com

Hiermit erkläre ich, dass ich mich noch keiner Doktorprüfung unterzogen oder mich um Zulassung zu einer solchen beworben habe.

Ich versichere, dass die Dissertation "*Causes and consequences of plant order of arrival on the structure and functioning of grassland communities*" in der gegenwärtigen oder einer anderen Fassung noch keiner anderen Hochschule zur Begutachtung vorgelegen hat.

Ich versichere an Eides statt, dass ich die eingereichte Dissertation "*Causes and consequences of plant order of arrival on the structure and functioning of grassland communities*" selbstständig und ohne zulässige fremde Hilfe verfasst habe. Anderer als der von mir angegebenen Hilfsmittel und Schriften habe ich mich nicht bedient. Alle wörtlich oder sinngemäß anderen Schriften entnommenen Stellen habe ich kenntlich gemacht. Über die strafrechtlichen Folgen gemäß § 156 Strafgesetzbuch wurde ich in Kenntnis gesetzt.

Lüneburg, 27 Nov 2023

Appendix 4

with regard to section 9 (2.4 and 5) as well as section 9 (3) “Opening of the Doctoral Procedure“ of the German version of the doctoral regulation of 2023

Statements and Declaration

Inés María Alonso-Crespo

ines.alonso_crespo@leuphana.de / inesalonsocrespo@gmail.com

I hereby declare that I have never taken any doctoral examination or applied for admission to such examination.

I further affirm that the dissertation with the title “*Causes and consequences of plant order of arrival on the structure and functioning of grassland communities*” has not been submitted to any representative of any school and that I am submitting the dissertation only in this and in no other doctoral procedure and that no other definitely fail has been achieved in any previous doctoral procedure.

I furthermore declare, that I composed the submitted dissertation “*Causes and consequences of plant order of arrival on the structure and functioning of grassland communities* ” independently and without having recourse to prohibited means. I have not used any aids or texts other than those I indicated. All passages taken in verbatim or substance from other works have been identified.

Lüneburg, 27 Nov 2023

Authorship declaration

Inés M. Alonso-Crespo **Causes and consequences of plant order of arrival on the structure and functioning of grassland communities**

Overview of articles included in the doctoral thesis

1. Alonso-Crespo, I. M., Temperton, V. M., Fichtner, A., Niemeyer T., Schloter M., & Delory, B. M. (*Submitted*) Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment. *Journal of ecology.* ID JEcol-2023-1025

2. Alonso-Crespo, I. M., Temperton, V. M., Fichtner, A., Schloter M., Gschwendtner, S., & Delory, B. M. (*In prep*.). Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment

3. Alonso-Crespo, I. M., Weidlich, E. W., Temperton, V. M., & Delory, B. M. (2023). Assembly history modulates vertical root distribution in a grassland experiment. Oikos, 2023(1), e08886.https://doi.org/10.1111/oik.08886

(in accordance with the Guideline for cumulative dissertations enacted at the Faculty of Sustainability in January 2012)

Explanatory Notes:

Articles included in a cumulative doctoral thesis can also be conference contributions or book chapters besides papers published in journals.

Bibliography: Author(s) – Title – Journal / Book / Conference contribution – Date of publication – DOI (if available)

Specific contribution of PhD candidate submitting the doctoral thesis / Author status according to § 12 of the guideline for cumulative dissertations

- Single author = own contribution amounts to 100%.
- Co-author with predominant contribution = wn contribution is greater than the individual share of all other co-authors and is at least 35%.
- Co-author with equal contribution = (1) own contribution is as high as the share of other co-authors, (2) no other co-author has a contribution higher than the own contribution, and (3) the own contribution is at least 25%.
- Co-author with important contribution = own contribution is at least 25%, but is insufficient to qualify as single authorship, predominant or equal contribution.
- Co-author with small contribution = own contribution is less than 20%.

Weighing Factor according to § 14 of the guideline for cumulative dissertations

Single author	1.0
Co-author with predominant contribution	1.0
Co-author with equal contribution	1.0
Co-author with important contribution	0.5
Co-author with small contribution	0

Article No.	Bibliography	Publication Status	Specific contribution	Weighing Factor
1	Alonso-Crespo, I. M., Temperton, V. M., Fichtner, A., Niemeyer T., Schloter M., & Delory, B. M. (*Submitted*) Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment. *Journal of ecology.* ID JEcol-2023-1025	Submitted	VMT, BMD and MS conceived the project, designed the experiment and secured funding; IMAC, BMD, TN and VMT collected data; IMAC and BMD analysed root images; IMAC, BMD and AF analysed data; IMAC produced the first draft of the manuscript, with support from BMD, AF and VMT. All authors contributed critically to the drafts and gave final approval for publication.	Co-author with predominant contribution (1.0)
2	Alonso-Crespo, I. M., Temperton, V. M., Fichtner, A., Schloter M., Gschwendtner, S., & Delory, B. M. (*In prep*.). Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment	In preparation	VMT, BMD and MS conceived the project, designed the experiment and secured funding; IMAC BMD and SG collected data; IMAC, BMD, SG and AF analysed data; IMAC produced the first draft of the manuscript. BMD, AF, VMT, MS and SG review and editing. All authors will contribute critically to the drafts and gave final approval for publication.	Co-author with predominant contribution (1.0)
3	Alonso-Crespo, I. M., Weidlich, E. W., Temperton, V. M., & Delory, B. M. (2023). Assembly history modulates vertical root distribution in a grassland experiment. Oikos, 2023(1), e08886.https://doi.org/10.1111/oik.08886	Published	IMAC: Formal analysis (equal); Investigation (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal). EWAWConceptualization (equal); Investigation (equal); Writing – review and editing (equal). VMT: Conceptualization (equal); Supervision (equal); Writing – review and editing (equal). BMD: 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).	Co-author with predominant contribution (1.0)

Authors: (AF) Andreas Fitchner, (BMD) Benjamin M. Delory, (EWAW) Emanuela Weidlich, (IMAC) Inés M. Alonso-Crespo, (MS) Michael Schloter, (SG) Silvia Gschwendtner, (TN) Thomas Niemeyer, (VMT) Vicky M. Temperton.

Declaration (according to § 16 of the guideline for cumulative dissertations)

I avouch that all information given in this appendix is true in each instance and overall.

Lüneburg, 27 Nov 2023

In adherence to the established rule, the inclusion of papers that have not yet received acceptance or publication in a peer-reviewed journal is justified based on their significant potential for scholarly contribution. Although not currently published or accepted, these articles have undergone rigorous evaluation through their presentation in international conferences characterized by open calls for papers and a stringent peer-review process. The decision to include them in the doctoral examination is grounded in the recognition of their merit and alignment with external quality indicators. The PhD examiners are entrusted with the task of assessing these articles, taking into account their potential for future publication in peer-reviewed journals widely recognized by the relevant scientific community. This approach aligns with the dynamic nature of academic research and the importance of recognizing valuable contributions that may not yet have reached the final stage of formal publication but have demonstrated promise and scholarly rigor through their exposure in reputable academic forums.

The conferences in which both works have been presented are listed below.

Article 1: Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment

Alonso-Crespo, I.M., Delory, B.M., de Kroon, H., Schloter, M., Weidlich, E.W.A.& Temperton, V.M. Priority Effects: mechanisms, strength and persistence over time in dry grasslands. Oral presentation. BES Annual Meeting (Bristish Ecological Society). Edinburgh, Scotland. December, 2022

Alonso-Crespo, I.M., Delory, B.M., de Kroon, H., Schloter, M., Weidlich, E.W.A.& Temperton, V.M. Priority effects in dry grasslands: insights from a field experiment and implications for restoration. Oral presentation in: 13th SERE (European Society for Ecological Restoration). Alicante, Spain. September 2022.

Alonso-Crespo, I.M., Delory, B.M., de Kroon, H., Schloter, M. & Temperton, V.M. POEM - PriOrity Effect Mechanisms: mechanisms of priority effects and their persistence over time in dry acidic grasslands. Oral presentation in: XV Congreso Nacional de la AEET (Asociación Española de Ecología Terrestre) - Plasencia, Cáceres, Spain. October 2021.

Article 2: Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment

Alonso-Crespo, I.M., Delory, B.M., Schloter, M., Weidlich, E.W.A.& Temperton, V.M. Disentangling the mechanisms creating priority effects in dry grasslands: a Plant-Soil Feedback experiment. Poster presentation. BES Annual Meeting (Bristish Ecological Society). Edinburgh, Scotland. December, 2022

Alonso-Crespo, I.M., Temperton, V.M., Schloter, M. & Delory, B.M. Disentangling the mechanisms creating priority effects in dry grasslands: a Plant-Soil Feedback experiment - Poster presentation in: Aboveground-Belowground Interactions (British Ecological Society), Marseille, France. May 2022.

Curriculum Vitae

PERSONAL INFORMATION

Name: Alonso–Crespo, Inés María

ORCID identifier:

https://orcid.org/0000-0002-4283-3639

Date of birth: 18 January 1991

Nationality: Spanish

Languages:

- Spanish (mother tongue)
- English (fluent)
- German (basic)

✉ inesalonsocrespo@gmail.com

Professional:

☎ ines.alonso_crespo@leuphana.de

Tel (professional):

+49 4131 677 2815

About me

I studied Environmental Sciences and did a master's degree focused on the biology and conservation of biodiversity at the University of Salamanca (USAL) in Spain. For several years, I collaborated with the Ecology department of this university, studying the regenerative characteristics of *Quercus faginea*.

In 2020, I started my PhD programme at Leuphana Universität Lüneburg in Germany, where I am currently investigating the impact of the order of arrival of different plant functional groups on above- and below-ground plant development at community and species level in dry grasslands, exploring also how climatic conditions during the establishment year modulate these effects in the POEM experiment (@POEM_experiment). In addition, I am investigating plant-microbiome and plant-soil interactions, analysing the mechanisms underlying this phenomenon.

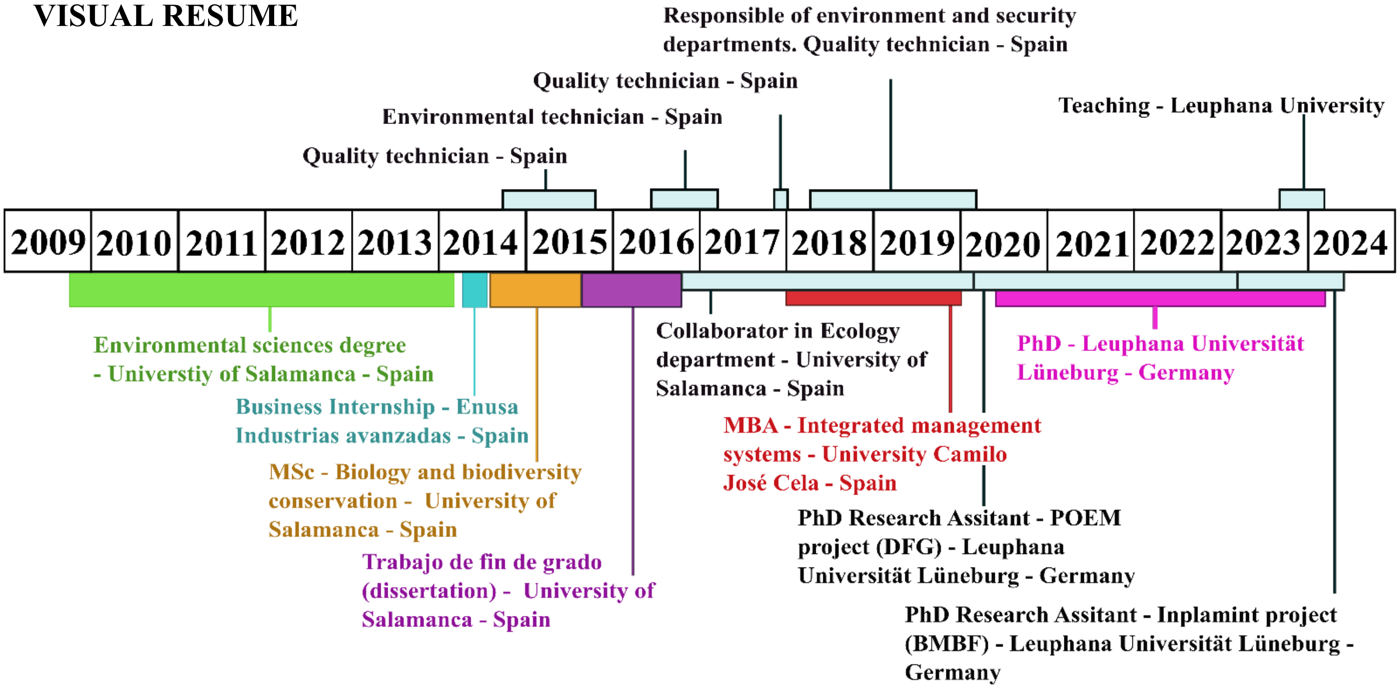
In 2023, I became involved in the InPlaMint project (https://www.bonares.de/inplamint-de), focusing on the combined effects of drought and high organic amendments on crop productivity and carbon and nitrogen allocation.

My main focus is on plant-plant and plant-soil interactions, but I am also very interested in how plants respond to human or natural disturbances. I am involved in other experiments that test the effects of microplastics on plant development and examine the impact of human population density and latitude on trophic interactions.

https://de.linkedin.com/in/in%C3%A9s-m-alonso-crespo-a352b495

https://twitter.com/inesacrespo

VISUAL RESUME



ACADEMIC FORMATION

- PhD – Sustainability Sciences
  Leuphana Universität Lüneburg – Lüneburg - Germany - 2020-currently
- MBA - Integrated Management Systems (8.41/10)
  Universidad Camilo José Cela - Madrid - Spain - 2018-2019
- Trabajo de fin de grado (Tesina – "small dissertation") (CUM LAUDE)
  Universidad de Salamanca – Salamanca – Spain - 2016
- MSc – Biology and biodiversity conservation (8.61/10)
  University of Salamanca - Salamanca - Spain - 2014-2015
- Business internship: Environmental Technician
  ENUSA Industrias Avanzadas S.A Juzbado - Salamanca - Spain - 2014
- Environmental sciences degree (6.24/10)
  University of Salamanca - Salamanca - Spain - 2009-2014

PROFESIONAL TRAJECTORY

- **Oct 2023 – Currently – Teaching: complementary studies –** Leuphana Universität Lüneburg
- **Mar 2023 - Currently – PhD RESEARCH ASSISTANT – INPLAMINT project-** Bundesministerium für Bildung und Forschung - BonaRes Projekten - Leuphana Universität Lüneburg - Lüneburg – Germany
  Experimental design, data acquisition, data analysis, scientific writing and publication, student's supervision and training.
- **Mar 2020 - 2023 – PhD RESEARCH ASSISTANT – POEM: Priority Effects mechanisms project-** Deutsche Forschungsgemainschaft (DFG) - Leuphana Universität Lüneburg - Lüneburg – Germany
  Experimental design, data acquisition, data analysis, scientific writing and publication, students training.
- **Mar 2018 - Feb 2020 - HEAD OF ENVIRONMENT AND HEALH AND SECURITY AND QUALITY TECHNICIAN** - Transervi S.A - Soto del Real- Madrid – Spain
  Audits. QA. Environment. Security. ISO-9001, ISO 14001, EMAS. Training and information to workers and clients. Teach methodologies and regulations. Procedure. Continuous improvement.
- **Sep 2016 - Jan 2020 - RESEARCH COLLABORATOR** - University of Salamanca - Salamanca – Spain
  Experimental design, data acquisition, data analysis, scientific writing and publication
- **Dec 2017 - QUALITY TECHNICIAN** - Moisés Alonso Martín S.L Guijuelo - Salamanca – Spain
  Audits. QA. Procedure. Continuous improvement.
- **Jun 2016 - Jan 2017 - QUALITY AND ENVIRONMENTAL TECHNICIAN** - ENUSA Industrias Avanzadas S.A Juzbado - Salamanca – Spain
  Audits. Environment. ISO-9001, ISO 14001, EMAS. Training and information to workers. Teach methodologies and regulations. Procedure. Continuous improvement.
- **Jun 2014 – Aug 2015 - QUALITY TECHNICIAN** - Moisés Alonso Martín S.L Guijuelo - Salamanca – Spain
  Audits. QA. Procedure. Continuous improvement.

PUBLICATIONS

- Alonso-Crespo, I.M., Silla, F., Jiménez del Nogal, P.M., Fernández, M.J., Martínez-Ruiz, C. & Fernández-Santos, B. (2020) Effect of the mother tree age and acorn weight in the regenerative characteristics of *Quercus faginea*. *European Journal of Forest Research*, *139*, 513-523. https://doi.org/10.1007/s10342-020-01266-8
- Alonso-Crespo, I.M., Weidlich, E.W.A., Temperton, V.M. and Delory, B.M. (2023) Assembly history modulates vertical root distribution in a grassland experiment. *Oikos e*08886. https://doi.org/10.1111/oik.08886
- Alonso-Crespo, I. M., & Hernández-Agüero, J. A. (2023). Shedding light on trophic interactions: A field experiment on the effect of human population between latitudes on herbivory and predation patterns. *Ecology and Evolution*, *13*(9), e10449. https://doi.org/10.1002/ece3.10449
- Alonso-Crespo, I.M., Delory, B.M., Fichtner A., Niemeyer, T., Schloter, M. & Temperton, V.M. (Submitted) Exploring priority and year effects on plant diversity, productivity and vertical root distribution: first insights from a grassland field experiment. *Journal of Ecology*. JEcol-2023-1025 pre-print: https://biorxiv.org/cgi/content/short/2023.11.14.566982v1
- Kumar, A., Kuznetsova, O., Gschwendtner S., Chen, H., Alonso-Crespo, I.M., Schulz, S., Bonkowski, M., Schloter, M., & Temperton, V.M. (ready for submission) Impact of soil microbial life on root and rhizosphere traits of wild and modern barley. Pre-print: https://www.authorea.com/users/637680/articles/653874-implications-of-domestication-syndrome-in-barley-for-above-and-belowground-plant-traits-and-microbial-interactions?commit=5e4600272c5bef43cbe3aa4e39b285454c931b4c
- Alonso-Crespo, I.M., Delory, B.M., Schloter, M., Gschwendtner S. & Temperton, V.M. (In prep.) Disentangling the mechanisms creating priority effects in dry grasslands: a plant-soil feedback experiment.
- Alonso-Crespo, I.M., & Mateos-Cárdenas, A. (In prep.) Presence of Bio and petroleum PE plastic particles affect root and shoot development: a test in *Bromus hordeaceus*.

FUNDED PROJECTS

2022 - Human presence impacts on bird predation across latitudinal gradient: a field experiment. In collaboration with Dr. Juan Antonio Hernández-Agüero. Funded by: Sociedad Española de Etología y Ecología Evolutiva (SEEEE)

Acknowledgements

Thank you, Vicky, for providing me with the opportunity to be here, and for your support throughout.

Ben, your guidance and mentorship have been invaluable, and I'm grateful for everything I've learned under your tutelage over these years.

Andreas thank you for getting involved in this thesis and offering your wise counsel.

To the three of you, thank you for your support and the wealth of knowledge you have shared with me.

Gracias a mis padres, Moisés y Elena, y a mi hermana, Alicia, por vuestro apoyo y confianza constante. Por todos sus consejos. Gracias por tejer una red tan fuerte que me permitió y permite tomar decisiones sin miedo.

Gracias a Juan, porque si no te hubieras cruzado en mi vida, probablemente no estaría hoy aquí. Gracias por creer siempre en mí y hacerme ver lo que valgo. Por animarme y levantarme a cada paso, por cada comentario. Por ser el otro *Leakey* de esta pareja.

Thanks to Thomas and Christoph for your invaluable help which has made my job much easier. And thank you very much for the barbecues and *Mälzer* dinners, which were my social life in 2020 and 2021.

Thanks Tanja, for being una gran persona y compañera de trabajo. Gracias por siempre ayudarme, profesional y personalmente.

Thanks to Amit Kumar for always being willing to lend a hand.

Gracias a mis abuelos, por llamarme cada semana y por volar por primera vez en su vida para acompañarme en este viaje.

Gracias a mis tías, tíos, primas y primos, por hacerme sentir siempre parte de la familia, a pesar de la distancia. Gracias a los que siempre estáis conmigo.

Gracias a mis amigos, para los que los km no fueron un obstáculo, que me vieron marchar y estarán cuando vuelva, "compartiendo pan de Lembas como Frodo y Samsagaz".

Gracias a Ángela, Merxe y Marta, por mantener mi cuerpo y mi mente en el lugar que merecen.

My sincere thanks to Dr. Alicia Mateos-Cárdenas and Dr. Alina Twerski, thank you for your review of this thesis, for your time and for your continued support. It is an honour to share a profession with women like you.

Thank you very much to Dr. Martí March Salas, for always answer my "early-career" questions and for taking the time of reviewing this thesis.

Thanks to Joana Bendler for reviewing the Zusammenfassung.

Thanks to all the people and colleagues who have shared this adventure with me in one way or another. For those who made me feel at home when I first arrived and for those who have made me feel part of something else. You have made this journey so much more enjoyable.

Thank you to all the students that have participated in the experiments of this thesis: Marlon Fiebing, Olga Kuznetsova, Anais Verstraeten, Rhea Helmerich, Lukas Van Treeck, Luzie Glock, Lena Thurich, Esther Vogt, Mónica Mercado, Fieke Grunwaldt, Johan Busse von Colbe, Katrin Süring, Marieke Scheel, Sepher Kashi, Milosz Rembowski, Julia Masjosthusmann, Sara Gerda Buss, Inês Quick, Olga Vishnyakova, Eylul Umay, Pia Volpert, Conrad Moosdorf, Lisa Herfurth, Maike Annika Truempler, Teresa Gross, Linde Koehne, Merle Eigemann and Nima Namjoo. Without your work, it would have not been possible.

I would also like to thank the German research system for giving me the opportunity that my country could not give me. Especially the Deutsche Forschungsgemeinschaft (DFG), which financed part of the experiments that today make up this thesis.

And last, but not least, to rephrase *Snoop Dog*, “I wanna thank me”. Thanks for being like a wicker rod that “bends rather than breaks" and enduring the process of immigration with the addition of the pandemic and its isolation. For staying even when nothing made sense anymore.

Look where you have come, today there is a little girl crying with happiness to see what you have achieved.

LEUPHANA
UNIVERSITÄT LÜNEBURG