


# Soil biota and chemical interactions promote co-existence in co-evolved grassland communities

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## Abstract

1. Plant populations can exhibit local adaptation to their abiotic environment, such as climate and soil properties, as well as biotic components such as the chemical signatures of dominant plant species and mutualistic and pathogenic microbial populations. While patterns of local adaptation in individual species are widely recorded, the importance of microevolutionary processes for plant community assembly and function is poorly understood.
2. Here, we examined how a history of long-term co-existence, and thus potential for local co-adaptation, influenced the process of plant community assembly. Soil inocula and seeds of eight plant species were collected from three calcareous grasslands with a long history of grazing within a single geographical region. Mesocosm communities were established using local genotypes from a single site or an artificial mixture of genotypes from two different sites. To investigate the role of root exudates and local ('home') and non-local ('away') soil biota as mediators of plant species co-existence, the population origin treatment was combined with the addition of activated carbon, which is known to adsorb exudates from soil, and sterilization of soil inocula. Individual-, species- and mesocosm-level responses were measured over the course of three growing seasons.
3. We found that root exudates promoted seedling survival, species co-existence and productivity in assemblages of genotypes originating from the same community but had a weak impact in mixed, novel communities. Soil biota promoted the growth of subordinate forbs and restrained the growth of dominant graminoids, particularly in communities composed of local genotypes. The effects of population origin were significant in the first 2 years of the experiment but were not detectable in the third year when interbreeding and new seedling establishment took place. Plant genotypes coupled with 'home' microbial inoculum experienced a stronger reduction in growth compared with genotypes exposed to 'away' inoculum, indicating that plants experienced home-field disadvantage in interactions with soil biota.
4. *Synthesis.* Our study demonstrates that the mechanisms of initial grassland community assembly depend on community history, with below-ground chemical

interactions and plant interactions with soil biota becoming stronger drivers of dynamics in established and potentially co-evolved communities.

#### KEYWORDS

below-ground interactions, community evolution, eco-evolutionary dynamics, grassland restoration, local adaptation, plant community assembly, root exudates, soil microbial interactions

## 1 | INTRODUCTION

Plant community assembly can be viewed as a process of species passing several abiotic and biotic filters, with local community composition determined by the ability of species to reach a particular geographic location and persist in a given set of environmental conditions (Kraft et al., 2015; Lortie et al., 2004; Myers & Harms, 2011; Zobel, 1997). However, plant populations continue to experience selective pressures from the abiotic and biotic components of the environment beyond the initial assembly of the community. Many studies have documented local adaptation within plant species in response to abiotic properties, such as climate and soil properties (Bischoff et al., 2006; Brady, Kruckeberg, & Bradshaw, 2005; Macel et al., 2007) as well as to co-occurring plant species, their diversity and chemical signatures (Abakumova, Zobel, Lepik, & Semchenko, 2016; Grøndahl & Ehlers, 2008; Thorpe, Aschehoug, Atwater, & Callaway, 2011). Plants also exhibit local co-adaptation with microbial species, including mycorrhizal and pathogenic fungi (Höckerstedt, Siren, & Laine, 2018; Johnson, Wilson, Bowker, Wilson, & Miller, 2010). While the adaptation of individual species to local environments is well documented, knowledge of how this influences community-level processes is still lacking.

The likelihood of local co-existence is determined by the extent of fitness differences and resource niche overlap among species, with co-existence most likely when species niches are sufficiently distinct to compensate for inherent differences in fitness (Cadotte, 2017; Lankau, 2011; Mayfield & Levine, 2010). However, recent theoretical and empirical studies suggest that evolution among competitors can lead to diverse outcomes. Reduced fitness differences between species and greater potential for co-existence may result from both niche convergence and divergence (Edwards et al., 2018; terHorst, Miller, & Powell, 2010). In artificially assembled plant communities, 12 years of selection in species mixtures resulted in positive effects on community productivity and divergence in some traits, indicating the evolution of niche differentiation, but also convergence in other traits, indicating evolution towards the optimum competitive phenotype (van Moorsel, Schmid, Hahl, Zuppinger-Dingley, & Schmid, 2018; Zuppinger-Dingley et al., 2014). Overall, a history of co-existence has been shown to result in reduced competitive and increased facilitative interactions in species-rich plant communities (Schöb, Brooker, & Zuppinger-Dingley, 2018; Semchenko, Abakumova, Lepik, & Zobel, 2013).

Beyond direct interactions based on resource consumption, plant co-existence and evolutionary dynamics are also strongly influenced by chemical interference and interactions mediated by soil

biota. Plants can exhibit allelopathic interactions, whereby plants produce toxic litter or root exudates that suppress the growth of neighbouring species. Such effects are typically observed in plant assemblages with limited history of co-existence, such as communities affected by invasive species or agricultural systems (Callaway, Ridenour, Laboski, Weir, & Vivanco, 2005; Kong et al., 2018; Lankau & Strauss, 2007). The toxicity of allelochemicals in the invaded range tends to decline with time, and such chemicals often have neutral or positive effects on neighbouring plants in the native range (Callaway & Aschehoug, 2000; Callaway & Ridenour, 2004; Huang, Lankau, & Peng, 2018; Nielsen, Grøndahl, Callaway, Dickinson, & Ehlers, 2017). Local adaptation to the chemical signatures of dominant species can also be detected within native communities. It has been shown that aromatic compounds produced by *Thymus* species exert a selective pressure on associated vegetation, resulting in higher frequencies of genotypes with positive growth responses to *Thymus* chemicals at sites dominated by *Thymus* compared with sites where it is absent (Jensen & Ehlers, 2010). Moreover, chemical conditioning of soils by *Thymus* enhances the species richness of associated vegetation in native, co-evolved communities but decreases diversity in the introduced range (Ehlers, Charpentier, & Grøndahl, 2014; Nielsen et al., 2017). Collectively, these studies demonstrate that chemical interactions can play a vital role in the process of local adaptation and may enhance species co-existence.

Local co-adaptation with microbial species has most often been recorded in host–pathogen systems (Kraemer & Boynton, 2017). Fungal pathogens exhibit local adaptation to their host plants such that local plant genotypes tend to experience a more severe infection from local pathogen strains than from pathogens lacking a history of co-evolution (Crémieux et al., 2008; Höckerstedt et al., 2018; Thrall, Burdon, & Bever, 2002). Plants also show different trajectories of co-evolution with mycorrhizal fungi on different soil types, with mutualistic interactions between plants and fungi maximized in phosphorus-deficient soils and parasitic interactions minimized in phosphorus-rich soils (Johnson et al., 2010; Pánková, Münzbergová, Rydlová, & Vosátka, 2008). During plant invasions and species range expansions, invading plants may experience a release from pathogen control while also disrupting local mutualistic interactions, with both processes providing an advantage in competition with resident plant communities (Engelkes et al., 2008; Koorem et al., 2018; Morriën, Engelkes, Macel, Meisner, & van der Putten, 2010).

While local adaptation has been widely recorded in individual plant species, the importance of evolutionary processes for plant

community assembly and function, and the roles of chemical and microbial interactions in mediating these processes, remains poorly understood. Predicting how the disruption of established relationships among plants will influence community processes is becoming particularly pertinent as species ranges are shifting due to climate change and human disturbance (Davis & Shaw, 2001; van Grunsven et al., 2007; Morriën et al., 2010; Olden, Poff, Douglas, Douglas, & Fausch, 2004). Similarly, restoration of lost habitats often involves mixtures of plant and microbial populations with no recent history of local co-existence, but the implications of such practices are poorly understood (Bischoff, Steinger, & Müller-Schärer, 2010; Bucharova et al., 2017; Crémieux, Bischoff, Müller-Schärer, & Steinger, 2010; Durka et al., 2017; Verhoeven, Macel, Wolfe, & Biere, 2011; Wubs, van der Putten, Bosch, & Bezemer, 2016).

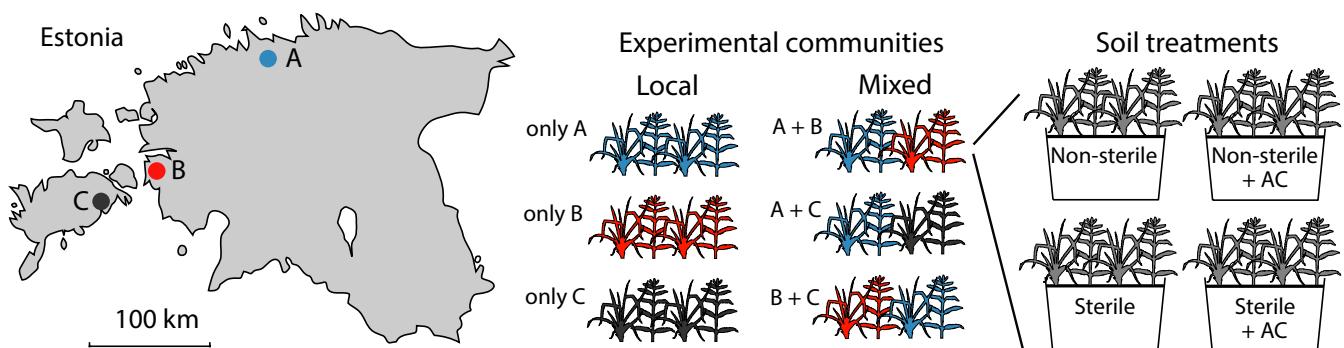
In this study, we experimentally manipulated the genotypic composition of model grassland communities to examine how a history of long-term co-existence, and thus potential for local co-adaptation, affects plant community assembly and shapes the mechanisms of species co-existence. Experimental communities were established using seed collected from ancient calcareous grasslands with a long history of grazing (at least 100 years). Communities were assembled from either exclusively local genotypes or from a mixture of genotypes from different communities. To determine the roles of soil microbial communities and root exudates in mediating plant community assembly, plants communities were inoculated with either a local, non-local or sterile soil microbial inoculum and soil in half of the communities was supplemented with activated carbon powder, which is known to absorb organic compounds from soil solution and interfere with below-ground chemical interactions (Callaway & Aschehoug, 2000; Callaway et al., 2005). We predicted that plant communities composed exclusively of local, potentially co-evolved genotypes would exhibit lower plant mortality rates, and higher levels of species co-existence and productivity compared with artificially mixed, novel communities. Plant root exudates and local soil microbial communities were predicted to promote species

co-existence in local plant communities but have a neutral or negative effect on co-existence in novel communities. Lastly, we predicted that non-local genotypes would experience a release from negative biotic interactions in mixed plant communities compared with local genotypes coupled with their “home” biota.

## 2 | MATERIALS AND METHODS

### 2.1 | Study species and growth conditions

Seeds and soil were collected from three calcareous grasslands in Estonia: Site A (59°21'16"N 24°59'02"E), Site B (58°38'32"N 23°30'56"E) and Site C (58°26'13"N 23°04'13"E), located 40–150 km apart from of each other (Figure 1). Seeds of eight perennial herbaceous plant species were collected from each site: *Campanula rotundifolia* L. (Campanulaceae), *Carex flacca* Schreb. (Cyperaceae), *Festuca ovina* L. (Poaceae), *Galium verum* L. (Rubiaceae), *Hypericum perforatum* L. (Hypericaceae), *Inula salicina* L., *Leontodon hispidus* L. and *Pilosella officinarum* F.W. Schultz et Sch. Bip. (Asteraceae). The species were chosen to represent a range of common graminoid and forb species at the study sites. The sites have similar vegetation composition, represented by the eight study species as well as other species common to calcareous grasslands including *Anthyllis vulneraria* L. (Fabaceae), *Asperula tinctoria* L. (Rubiaceae), *Filipendula vulgaris* Moench (Rosaceae), *Galium boreale* L. (Rubiaceae), *Helictotrichon pratense* (L) Pilg. (Poaceae), *Juniperus communis* L. (Cupressaceae), *Linum catharticum* L. (Linaceae), *Thymus serpyllum* L. (Lamiaceae) and *Trifolium montanum* L. (Fabaceae). The grasslands represent the same community type, but probably vary in the relative abundances of different species reflecting local environmental filtering and historical differences in establishment and management over the centuries (Gazol et al., 2012). The soil type in all grasslands is rendzic leptosol, that is, the soil profile contains only two horizons: humus, 2–25 cm thick (pH range at examined sites 7–8, total N range 4–7 g/kg, total P range 0.5–0.8 g/kg) and parent material of limestone mixed



**FIGURE 1** Experimental design. Soil and seeds of eight plant species were collected from three calcareous grasslands in Estonia (sites A, B and C). The seeds were used to establish two community types: local communities composed of genotypes from a single site only and mixed communities composed of a mixture of genotypes from two sites. Each of the resulting six plant communities was subjected to a factorial combination of two soil treatments: soil inoculation (sterile soil inoculated with either live or sterilized soil inoculum) and addition of activated carbon (soil with or without the addition of activated carbon powder, AC). In the local communities, soil inoculum and seeds originated from the same site; in the mixed communities, an inoculum from a single site was added to each pot so that half of the seedlings were coupled with their “home” and the other half with an “away” inoculum [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

with coastal sediments (Pärtel, Kalamees, Zobel, & Rosen, 1999). All grasslands have a long history of grazing (at least 100 years by sheep, cattle and horses) and have developed high small-scale vascular plant species richness (on average 27 species/m<sup>2</sup>; Pärtel et al., 1999; Wilson, Peet, Dengler, & Pärtel, 2012).

Surface-sterilized seeds were germinated in a greenhouse (ambient conditions) on moist, sterilized sand. Upon germination, the seedlings were transferred to a common garden and two seedlings of each species were transplanted into 3.5 litre pots (19 cm in diameter, 14.5 cm in height) containing a soil and sand mixture (see details below), resulting in 16 seedlings per pot in total. Seedling positions in pots were randomly assigned, with the restriction that seedlings were at least 2 cm apart from each other and 3 cm away from the edge of the pot. Seedling positions and identities were marked with coloured pins to enable monitoring of seedling survival over time. In the first three weeks of the experiment, dead seedlings were replaced with conspecifics originating from the same community or, in cases when no more conspecific seedlings were available, a seedling of a different species from the same community was used. As a result, species composition varied slightly between pots, but the plant population origin remained constant, and no significant biases in plant community composition were introduced in relation to the community type, activated carbon and soil inoculation treatments described in the experimental design below (Table S1).

Pots were maintained in the common garden (Tartu, Estonia; mean annual temperature: 5.8°C, mean annual precipitation: 680 mm) where plants were exposed to natural precipitation and received additional watering when necessary. Pot positions were randomized each month. The experiment lasted for three growing seasons (between June 2012 and August 2014). No fertilizer was applied; non-target species were regularly removed by weeding while allowing the regeneration of target species by clonal propagation (primarily in *C. flacca*, but also *P. officinarum* and *G. verum*) in the second and third years of the experiment and by seed (all species flowered by the end of the experiment). Mature Asteracea seeds, which are specifically adapted to disperse by wind, were manually collected and placed on the soil surface of “home” pots to prevent wind dispersal. All species survived to the end of the experiment, though there were local extinctions at the pot level. Overall, species diversity increased over time as a result of graminoid dominance decreasing and forb abundance increasing (Figure S1).

## 2.2 | Experimental design

Plants were subjected to a factorial combination of three treatments: community type, activated carbon addition and soil inoculation (Figure 1). Plants were grown in either local or mixed communities. In local communities, all seedlings originated from seed collected from the same site (three unique sites); in mixed communities, one replicate individual of each species originated from one site and the other replicate of each species from a different site (three pairwise combinations of sites). Hence, six unique communities were created in total. All pots (3.5 L) were filled with a mixture of fine sand (grain size < 0.2 mm)

and soil (rendzic leptosol) brought from calcareous grassland in North Estonia (a different site from the three sites used for seed and inoculum collection), at a ratio 1:2. The soil mixture was sterilized by gamma irradiation (dose 15 kGy). Each pot received 200 g of natural soil inoculum, which was either left intact (non-sterile inoculum) or was sterilized by gamma irradiation before adding to the pots (sterile inoculum). The origin of soil inoculum varied depending on plant community origin. In the local community treatment, the origin of soil inoculum was matched to the origin of the plant community. In the mixed community treatment, soil inoculum from one of the sites was added to each pot such that half of the seedlings in that pot experienced “home” soil biota and the other half of the seedlings experienced unfamiliar soil biota (“away” inoculum). Among the ten replicate pots of each mixed community, five pots received the inoculum from one site and five pots from the other site. Therefore, mixed communities simulated a situation where the local community is invaded by distant plant populations with no history of co-existence (e.g. as a result of habitat restoration with seed collected from elsewhere).

The soil inoculation treatment was combined with the addition of activated carbon: half of the pots received no activated carbon and half of the pots were filled with substrate that was mixed with activated carbon powder at the rate of 8 g per pot (resulting in a ratio 1:420; Compo, Münster, Germany). Activated carbon was reapplied every spring at the same rate per pot in the form of a water suspension. Activated carbon is known to adsorb organic compounds from soil solution and has been used in previous studies to remove root exudates from the substrate, diminishing their impact on plant–plant interactions (Callaway & Aschehoug, 2000; Callaway et al., 2005). As such, activated carbon is a tool to manipulate chemical interactions in the soil, including those mediated directly by exudates released by live roots as well as indirect interactions involving modification of exudates by microbial communities (Cipollini, Rigsby, & Barto, 2012). There were ten replicates of each combination of community type × activated carbon × soil inoculum, resulting in 240 pots in total.

Plant shoots were cut at 1 cm height in September 2012. Shoot harvesting was repeated at the end of July in 2013 and 2014. Plants were sorted to an individual at the first harvest and to species at the second and third harvests (separation of individuals was impossible due to clonal and seed propagation), were oven-dried at 75°C for 48 hr and weighed.

To estimate below-ground biomass production and root morphology, two cylindrical soil cores were taken per pot (diameter 2 cm, depth 14.5 cm) at the end of the first growth season (September 2012). The cores were positioned randomly in the central area of the pot (at least 3 cm away from the pot edge) and avoiding rooting points of any of the plants. After the removal of the soil cores, the gaps were filled with the same soil mixture as used at the start of the experiment. The roots were washed, scanned using Epson Perfection V700 PHOTO scanner and analysed with WinRhizo 2008a software (Regent Instruments Inc., Quebec, Canada) to measure root diameter and length. Root biomass was dried for 48 hr at 75°C and weighed. Specific root length (SRL) was calculated as the ratio of root length to root dry mass.

## 2.3 | Statistical analysis

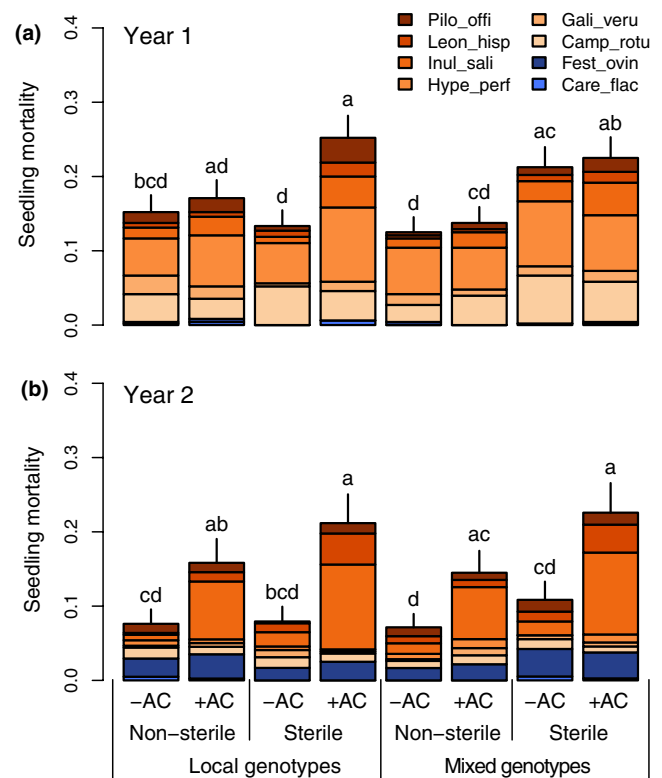
Plant mortality was analysed using generalized linear mixed effects (glmer) models with binomial error distributions, plant community composition (six levels: three communities composed of genotypes from a single site and three mixtures) and pot identity as random factors, and community type (local or mixed), activated carbon addition, inoculation (sterile or unsterilized soil inoculum) and all interactions as fixed factors. Plant mortality was only analysed in the first 2 years of the experiments as seedling recruitment in the third year made it impossible to accurately estimate the mortality of original individuals. Community biomass and diversity, and root morphology were analysed using linear mixed models (lmer) with  $\log_e$ -transformed biomass, root diameter, specific root length, or exponential Shannon index (i.e. effective species number, with eight being maximum if all species were evenly represented), as a response variable, community composition (six levels) as a random factor, and community type (local or mixed), activated carbon and soil inoculation (sterile or unsterilized soil inoculum) treatments and all interactions as fixed factors. In the mortality analysis, species was included in the model as an additional random factor. Separate models were performed for data from each year of the experiment.

To assess the home soil advantage or disadvantage on plant biomass and mortality, data from communities composed of population mixtures (mixed community type) were used. Individuals within each pot were split into plants that were exposed to their home soil or soil from an unfamiliar site, and generalized linear mixed models were performed with community composition (three population mixtures) and pot identity as random factors, and activated carbon, soil inoculation (sterile or unsterilized soil inoculum), soil inoculum origin (home or away) and all interactions as fixed factors. This analysis was only performed on the data from the end of the first growing season, as clonal spread of some individuals did not allow reliable tracing of the origin of each individual in subsequent years. All analyses were performed in R version 3.4.0 (R Core Team, 2017).

## 3 | RESULTS

### 3.1 | Seedling mortality

Some species experienced highest mortality in the first year of the experiment, while the mortality of other species was more pronounced in the second year (Figure 2). In the first year, the application of activated carbon caused 1.9 times higher seedling mortality in plant communities assembled from local plant genotypes and planted on sterile soil (Figure 2a). The application of activated carbon did not significantly impact mortality in communities composed of mixed populations (Figure 2a; interaction between community type and activated carbon  $\chi^2 = 5.4$ ,  $p = 0.021$ ; interaction between community type and inoculation  $\chi^2 = 5.8$ ,  $p = 0.016$ ; three-way interaction  $\chi^2 = 3.7$ ,  $p = 0.054$ ; Table 1). In the second year, seedling mortality was significantly higher in sterile soil ( $\chi^2 = 11.4$ ,  $p < 0.001$ ) and soil treated with activated carbon ( $\chi^2 = 61.5$ ,  $p < 0.001$ ), but



**FIGURE 2** The proportion of seedlings that did not survive by the end of (a) the first and (b) the second growth season of the experiment in plant communities composed of local genotypes or a mixture of genotypes from two distinct populations and subjected to the addition of activated carbon (AC) or soil biotic inoculum (sterile soil inoculated with unsterilized or sterilized inoculum). Species are represented by different shades and are stacked in the order in which they are listed in the key (six forbs at the top; two graminoids at the bottom). Standard errors of mean mortality are shown and different letters indicate significant differences in total mortality ( $p < 0.05$ ; Tukey's test). Species abbreviations: Pilo\_offi, *Pilosella officinarum*; Leon\_hisp, *Leontodon hispidus*; Inul\_sali, *Inula salicina*; Hype\_perf, *Hypericum perforatum*; Gali\_veru, *Galium verum*; Camp\_rotu, *Campanula rotundifolia*; Fest\_ovin, *Festuca ovina*; Care\_flac, *Carex flacca* [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 1** The effects of community type (local genotypes or mixed populations), addition of activated carbon and soil inoculation on plant mortality in the first 2 years of the experiment.  $\chi^2$ -statistics and their significance are shown

	Year 1	Year 2
Community type (T)	0.04	0.3
Carbon (C)	9.9**	61.5***
Inoculation (I)	22.2***	11.4***
T × C	5.4*	0.3
T × I	5.8*	1.6
C × I	3.0\$	0.6
T × C × I	3.7\$	0.2

\$ $p < 0.1$ .

\* $p < 0.05$ .

\*\* $p < 0.01$ .

\*\*\* $p < 0.001$ .



mortality rates were no longer affected by community type (Table 1, Figure 2b).

### 3.2 | Above-ground productivity and species diversity

Soil inoculation and activated carbon treatments had interactive and lasting effects on above-ground productivity across the 3 years of the experiment: the addition of activated carbon generally reduced plant productivity in soils inoculated with live soil biota but the effect on productivity was neutral or even slightly positive in soils that were sterilized using Gamma irradiation (Table 2, Figure 3). Moreover, communities composed of local genotypes were more sensitive to activated carbon addition in the first year of the experiment compared with communities composed of mixed populations ( $F_{1,228} = 7.9$ ,  $p = 0.005$ ; Table 2; Figure 3a). The differential effect of activated carbon on communities of different composition was primarily driven by changes in graminoid growth: activated carbon application significantly reduced the growth of graminoids in communities composed of local genotypes and inoculated with live local soil biota, while the growth of graminoids in communities with mixed populations was not significantly affected (three-way interaction  $F_{1,228} = 4.4$ ,  $p = 0.036$ ; Figure 3a). In the second year of the experiment, the soil inoculation treatment had an opposing effect on the growth of graminoids and forbs: graminoids benefited from soil sterilization and forb growth suffered from sterilization, particularly when communities were assembled from local genotypes and inoculated with local biota (interactive effects of community type and inoculation  $F_{1,228} = 6.7$ ,  $p = 0.010$  and  $F_{1,228} = 5.3$ ,  $p = 0.022$  for graminoid and forb biomass, respectively; Table 2, Figure 3b). The effects of activated carbon and soil inoculation persisted through to the third year of

the experiment but became less dependent on community type (Table 2, Figure 3c).

In addition to their effects on productivity, the application of activated carbon and the sterilization of soil inocula caused significant and additive declines in plant species diversity (Figure S2). The effect of activated carbon persisted through time while the effect of soil inoculation treatment even increased with the duration of the experiment and did not depend on community type (both effects significant at  $p < 0.005$ ; Table S2, Figure S2).

### 3.3 | Below-ground biomass and root traits

The application of activated carbon significantly reduced root production in communities composed of local genotypes but not in communities composed of mixed genotypes (interaction between the effects of community type and activated carbon  $F_{1,228} = 8.4$ ,  $p = 0.004$ ; Table 3, Figure 4a). The addition of activated carbon triggered the production of finer roots in communities of local genotypes and slightly increased root diameter in communities of mixed origin (interaction between the effects of community type and activated carbon  $F_{1,228} = 6.1$ ,  $p = 0.014$ ; Table 3, Figure 4b). In contrast to the differences observed in above-ground biomass, no significant effects of soil inoculation on below-ground biomass or root morphology were detected (Table 3).

### 3.4 | Effects of soil inoculum origin in mixed communities

Soil inoculation had a significant effect on seedling mortality, with lower mortality observed in soil inoculated with live biota, independent of whether plant genotypes were exposed to their "home" or "away" biota ( $\chi^2 = 27.0$ ,  $p < 0.001$ ; Table S3, Figure 5a). Plant

**TABLE 2** The effects of community type (local genotypes or mixed populations), addition of activated carbon and soil inoculation on total plant shoot mass per mesocosm in 3 years of the experiment

	Total shoot mass (g)			Graminoid shoot mass (g)			Forb shoot mass (g)		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Community type (T)	0.2	0.03	1.9	0.1	0.0	0.1	0.1	0.2	0.1
Carbon (C)	16.1***	0.1	4.5*	4.1*	0.1	5.3*	20.8***	5.5*	7.1**
Inoculation (I)	11.0**	43.8***	7.0**	24.7***	54.7***	41.4***	12.3***	17.6***	17.3***
T × C	7.9**	1.8	0.6	7.3**	0.5	0.0	0.0	0.6	0.1
T × I	3.8 <sup>§</sup>	3.0 <sup>§</sup>	0.1	4.4*	6.7*	2.2	0.1	5.3*	1.8
C × I	6.6*	5.1*	7.0**	3.5 <sup>§</sup>	1.2	6.0*	0.6	0.5	0.04
T × C × I	1.5	3.7 <sup>§</sup>	0.9	4.4*	0.4	0.2	4.1*	3.8 <sup>§</sup>	0.6

Note: F-values and their significance are shown. The analysis was performed on the dry mass of the whole community and of graminoids and forbs separately.

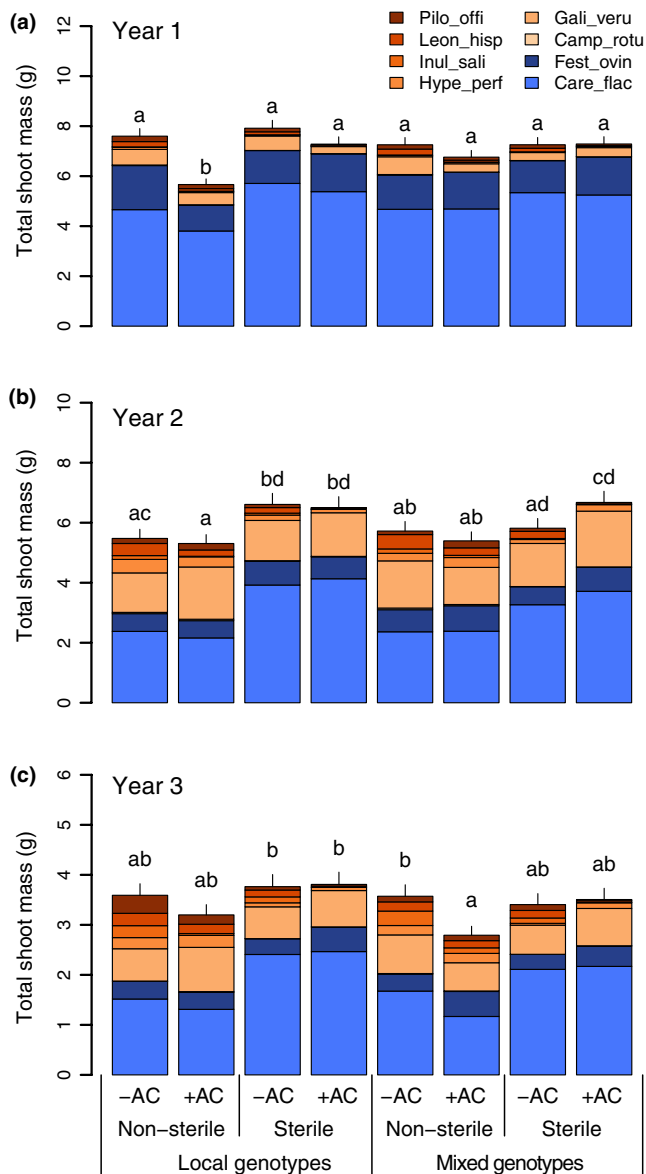
Degrees of freedom are 1 and 228 for numerator and denominator, respectively, except for graminoid biomass in the second and third year of the experiment where it was 227 and 226.

<sup>§</sup> $p < 0.1$ .

\* $p < 0.05$ .

\*\* $p < 0.01$ .

\*\*\* $p < 0.001$ .



**FIGURE 3** Total shoot dry mass in (a) the first, (b) second and (c) third year of the experiment in plant communities composed of local genotypes or a mixture of genotypes from two distinct populations and subjected to the addition of activated carbon (AC) or soil biotic inoculum (sterile soil inoculated with unsterilized or sterilized inoculum). Species are represented by different shades and are stacked in the order in which they are listed in the key (six forbs at the top; two graminoids at the bottom). Standard errors of mean biomass are shown and different letters indicate significant differences between means ( $p < 0.05$ ; Tukey's test). See Figure 2 for species abbreviations [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

above-ground productivity depended on the match between plant genotype and the origin of soil inoculum: plant genotypes exposed to "home" soil biotic inoculum were less productive than genotypes exposed to "away" soil biotic inoculum ( $F_{1,116} = 7.2$ ,  $p = 0.008$ ; Table S3, Figure 5b). In the absence of soil sterilization and activated carbon addition, plant genotypes exposed to "away" soil inoculum were 25% more productive than genotypes coupled with home soil (Figure 5b).

**TABLE 3** The effects of community type (local genotypes or mixed populations), addition of activated carbon and soil inoculation on root dry mass and root morphology at the end of the first growing season

	Root mass (g)	Root diameter (mm)	Specific root length (cm/mg)
Community type (T)	0.6	1.2	2.3
Carbon (C)	18.8**	1.3	0.3
Inoculation (I)	0.7	1.1	0.1
T × C	8.4**	6.1*	1.8
T × I	1.3	0.03	1.3
C × I	<0.01	0.8	3.3 <sup>\$</sup>
T × C × I	0.5	1.0	0.1

Note: F-values and their significance are shown.

Degrees of freedom are 1 and 228 for numerator and denominator, respectively, except for community type where the denominator degrees of freedom are 4.

\* $p < 0.05$ .

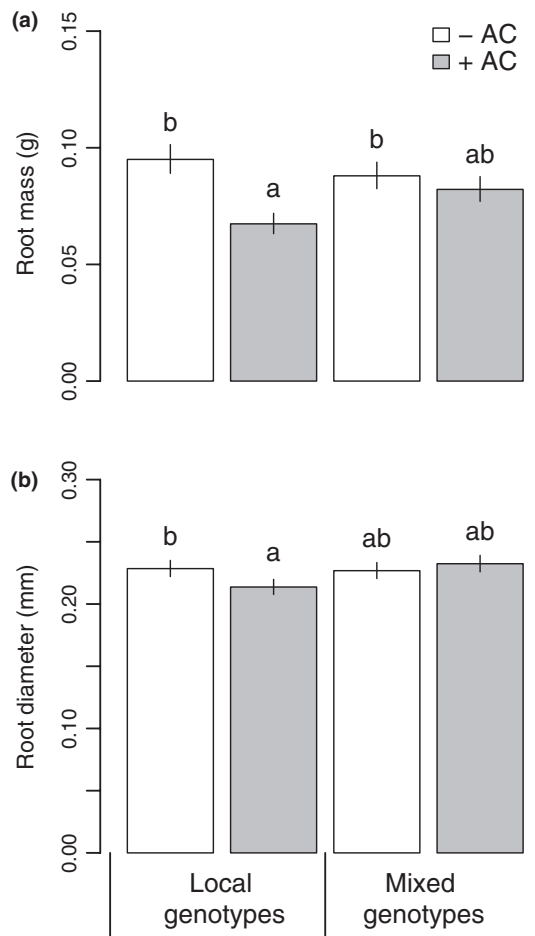
\*\* $p < 0.01$ .

<sup>\$</sup> $p < 0.1$ .

## 4 | DISCUSSION

Plant community assembly is a complex process that can involve not only ecological but also evolutionary processes, such as local adaptation to abiotic and biotic components of the environment (Bischoff et al., 2006; Grøndahl & Ehlers, 2008; Johnson et al., 2010; Macel et al., 2007). Such processes may fundamentally change the relative importance of resource competition and biotic interactions in structuring early versus late successional communities. Here, we show that below-ground chemical and microbial interactions become important mechanisms of community assembly in grasslands that experienced centuries of grazing management. We found that the disruption of below-ground interactions, by eradicating soil biota and adsorbing organic compounds from soil solution, had a negative effect on productivity, seedling survival and co-existence of different growth forms in communities of local genotypes, particularly in the first 2 years of community assembly, but such effects were absent in novel, artificially mixed communities. Moreover, plant genotypes that were exposed to "home" soil biota experienced suppressed growth compared with genotypes coupled with "away" soil biota, suggesting that soil biota with negative effects on plant growth, such as fungal pathogens (Semchenko et al., 2018), play an important role in controlling plant community assembly in ancient grasslands.

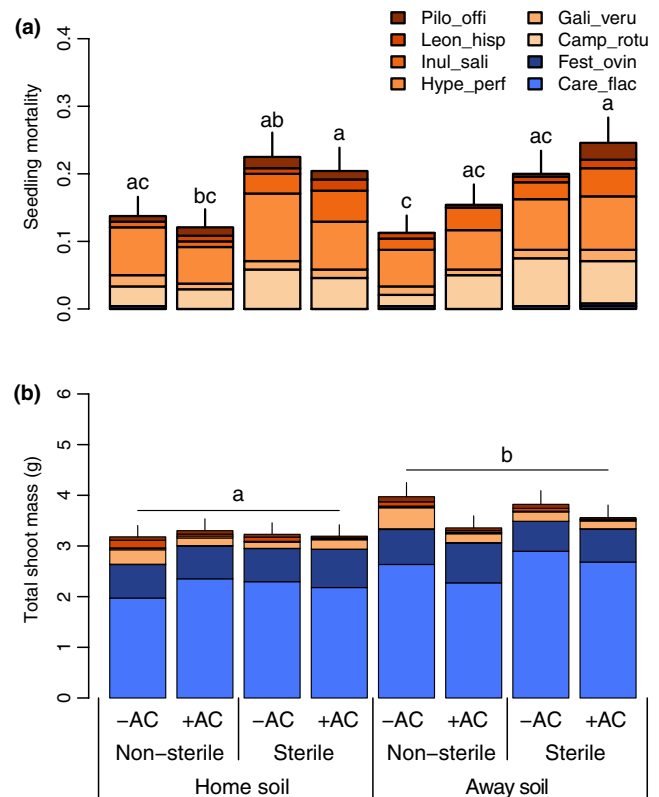
The addition of activated carbon to remove organic compounds, such as plant and microbial exudates, increased plant mortality and reduced productivity in both communities composed of local and mixed-origin genotypes. In the first year of community assembly, such effects were more pronounced in communities of local genotypes. These findings indicate that root exudates play an important role in mediating interactions between genotypes with a long history



**FIGURE 4** Root dry mass (a) and mean root diameter (b) in plant communities composed of local genotypes or a mixture of genotypes from two distinct populations subjected to the addition of activated carbon (AC; open bars without carbon addition and grey bars with carbon addition). Means ( $\pm 1SE$ ) are shown. Different letters indicate significant differences between means ( $p < 0.05$ ; Tukey's test) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

of co-existence, enhancing their establishment and growth at the seedling life stage. Interestingly, the growth of graminoids was reduced in the presence of activated carbon only when soil was inoculated with soil biota. This suggests that, in co-evolved plant-soil settings, graminoids may rely on root exudation to regulate interactions with potentially suppressive soil biota (Baetz & Martinoia, 2014).

We found that, in communities of local genotypes, plants produced finer roots in response to activated carbon addition. Plant and microbially derived exudates are known to strongly contribute to soil nutrient availability (Dakora & Phillips, 2002), and a switch to finer root production may be a compensatory response to nutrient limitation caused by the removal of exudates (Kramer-Walter & Laughlin, 2017). It has been shown that plant and microbial populations adapt to the chemical profiles of dominant plant species in the community, contributing to eco-evolutionary dynamics and species co-existence (Callaway et al., 2005; Ehlers & Thompson, 2004; Grøndahl & Ehlers, 2008; Huang et al., 2018; Jensen & Ehlers, 2010). Such effects are



**FIGURE 5** Total shoot dry mass (a) and mortality (b) of plants that were exposed to soil inoculum that originated from their home site or a different site from the same region (away soil). Mortality was calculated as the proportion of seedlings that did not survive by the end of the first growth season. Plants were grown in a mixed community where half of all individuals were exposed to the "home" soil inoculum and half to the "away" soil inoculum. The communities were established on sterilized soil that was either inoculated with sterilized or unsterilized inoculum and that was or was not subjected to the addition of activated carbon (AC). Species are represented by different shades and are stacked in the order in which they are listed in the key (six forbs at the top; two graminoids at the bottom). Standard errors of mean biomass and mortality are shown and different letters indicate significant differences between means ( $p < 0.05$ ; Tukey's test). See Figure 2 for species abbreviations [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

primarily demonstrated in comparisons between native systems and cross-continent invasions (Lankau & Strauss, 2007; Nielsen et al., 2017), but our results suggest that chemical interactions can exhibit a signature of local adaptation even in comparisons among native, local populations.

Inoculation with soil biota had a positive effect on plant species co-existence by reducing the dominance of graminoids and enhancing the growth of forbs. Therefore, graminoids exhibited net negative interactions with soil biota, while forbs experienced net positive interactions. The dominant graminoid in our study (*Carex flacca*) is generally poorly colonized by mycorrhizal fungi (Johnson et al., 2004), while three forb genera used in the study (*Inula*, *Leontodon* and *Pilosella*) are known to have obligate mycorrhizal associations (Gerz, Bueno, Ozinga, Zobel, & Moora,



2017). This confirms previous observations that species with low reliance on mycorrhizal fungi have net negative interactions with soil biota (Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn, 2016; Semchenko et al., 2018), and if such species tend to be most competitive in their resident communities, the negative impact of soil biota could enhance species co-existence (Heinze, Bergmann, Rillig, & Joshi, 2015; Lekberg et al., 2018). Here we show that such regulatory effects of soil biota significantly affected the first 2 years of plant community assembly and were particularly pronounced in communities comprised of local plant genotypes and local soil inoculum. Therefore, interactions with below-ground pathogens and mutualists become a stronger driver of seedling establishment and co-existence in ancient grassland communities compared to communities established from genotypes with no previous history of co-evolution.

In addition to stronger effects of soil biota in communities composed of local genotypes, we found a home-field disadvantage in mixed communities. We found that plant genotypes interacting with "home" biota showed reduced productivity compared with genotypes within the same community that were exposed to "away" biota. This finding agrees with studies on local adaptation in host-pathogen systems where pathogens tend to be ahead of their hosts in the evolutionary arms race and hence cause more damage to local plants (Höckerstedt et al., 2018; Kraemer & Boynton, 2017). As a result, plants can experience below-ground enemy release and become invasive when introduced to a new continent or as a result of climate-driven range expansion (Blumenthal, Mitchell, Pyšek, & Jarošík, 2009; Engelkes et al., 2008; van Grunsven, van der Putten, Bezemer, Berendse, & Veenendaal, 2010; Mitchell & Power, 2003). Our experimental manipulations suggest that similar processes may operate even within a limited geographic area, and the introduction of new genotypes into a local community, for example, in the process of habitat restoration, could significantly modify community dynamics.

Differential effects of activated carbon and soil inoculation on plant mortality and productivity in local versus mixed communities were pronounced in the first and second years of the experiment but were not detectable in the third year. Therefore, community origin may be important during initial community establishment but have transient effects in the longer term. In addition to these community-level effects, we detected a significant home-field disadvantage for local genotypes in mixed communities in the first year of the experiment. Further studies are necessary to establish how initial local genotype disadvantage affects plants when they reach the reproductive stage. Differential growth and reproductive success of local versus foreign genotypes could lead to significant shifts in the genetic composition of communities and erosion of local gene pools.

It is known that soil sterilization and activated carbon can affect soil nutrient availability (Brinkman, van der Putten, Bakker, & Verhoeven, 2010; McNamara, Black, Beresford, & Parekh, 2003; Semchenko, Zobel, Heinemeyer, & Hutchings, 2008; Weißhuhn & Prati, 2009). All treatments were established in sterilized soil and only a small quantity

of live soil was introduced as an inoculum, minimising nutrient differences between sterile and inoculated treatments. We also found significant interactive effects between activated carbon addition and genotypic composition of plant communities, suggesting that the effects of activated carbon were not merely due to generic interference with nutrient availability. Further insights could be gained by combining the manipulations of below-ground processes used in this study with sequencing of soil microbial communities, metabolomic profiling of root exudates and measurements of soil nutrient dynamics.

In conclusion, our study demonstrates that below-ground chemical interactions and plant interactions with soil biota play an important role in initial plant community assembly, affecting community productivity, seedling survival and species co-existence. Importantly, our data suggests that plant community assembly is more dependent on such interactions in grasslands with a long, stable history. These findings have important implications for nature conservation decisions and contribute to the current debate on the importance of locally sourced genetic material in restoration (Bucharova, 2017; Crémieux et al., 2010; Verhoeven et al., 2011). It seems that historical grassland habitats may be maintained by a fine-tuned network of interactions that can be disrupted through introduction of new genotypes. Re-creation of such habitats on degraded land may require many decades if not hundreds of years to allow co-adaptation or could be enhanced by the introduction of co-evolved plant and microbial assemblages.

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## AUTHORS' CONTRIBUTIONS

M.S. and K.Z. designed the study; M.S., S.N., A.S., M.A., R.K., A.L., K.P., S.S., M.S., M.T., Q.Z. and K.Z. carried out the study; M.S., J.D. and S.N. analysed the data; M.S. and S.N. wrote the first draft of the manuscript and all authors contributed to revisions.

## DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.5800rk5> (Semchenko et al., 2019).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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