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ORIGINAL PAPER

Time since invasion is not associated with the competitive effects of an alien herb

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Abstract Whilst temporal dynamics of the abundance of invasive alien species has gained interest, less is understood about complex interactions determining their impacts on, e.g., how coexistence mechanisms and the resulting ecological impact of alien species change with time. Using the invasive nitrogen-fixing *Lupinus polyphyllus* (lupine) and its native competitor *Leucanthemum vulgaris* (daisy), we investigated the effect of time since invasion on the growth of both plants, including lupine's allelopathic effects on daisy. We set up a full-factorial greenhouse experiment that utilised activated carbon to neutralise putative allelopathic compounds in the soil in relation to non-treated soils. The used field soils with natural microbial communities and abiotic soil properties were exposed to lupine for ca. 5 years, 10 years or 15 years, or had no known prior exposure (controls). Lupine significantly suppressed the growth of daisy in the first month and its final biomass. However, lupine's allelopathy did not appear to be the cause for the suppression of daisy seedlings that were grown next to lupine for two months. In addition, while the biomass of daisy benefitted from growing in previously lupine-invaded soils, both daisy and lupine, and their coexistence were not affected by the time soil was exposed to lupine. These results demonstrate that while time since invasion could be important for ecological impacts of some invasive alien plants or in older invasions, the suppressive effect of the invasive lupine on 1–3-month-old daisy was immediate and stable.

Keywords Time since invasion · Soil legacies · Invasion history · Invasive plants · *Lupinus polyphyllus* · Competition

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Introduction

Temporality is fundamental to many hypotheses testing the spread of invasive alien species and their impacts, thus leading to numerous calls for considering invasion history and time since invasion (Simberloff and Gibbons 2004; Strayer et al. 2006, 2017; Inderjit et al. 2011; Strayer 2012; Ricciardi et al. 2013; Crystal-Ornelas and Lockwood 2020a). However, temporal aspects of species invasions have been understudied (Iacarella et al. 2015; Grove et al. 2017a, but see e.g., Lankau et al. 2009; Diez et al. 2010; Dostál et al. 2013) with over half of articles on invasion biology not reporting or considering time since introduction or invasion (Crystal-Ornelas and Lockwood 2020a). Compared to temporal changes in the abundance of invasive plants, less is known about their ecological impacts at different invasion stages (Dostál 2024). Studies across taxa, including plants, suggest that the ecological impact of invasive alien species might weaken (Iacarella et al. 2015; Závorka et al. 2018), remain constant (Yokomizo et al. 2017; Crystal-Ornelas and Lockwood 2020b; Prass et al. 2022), or increase with time since invasion (Dostál 2024). Here we conducted a greenhouse experiment to examine whether time since invasion affects the competitive impact, or more specifically, the immediate allelopathy and soil legacies of the invasive legume, *Lupinus polyphyllus* (garden lupine), on growth of the native plant, *Leucanthemum vulgare* (ox-eyed daisy).

Dostál (2024) recently reviewed 19 study systems of invasive plants with long-term data or the space-for-time approach, which highlighted that multiple processes were involved in determining outcomes and temporal change within coexistence between species, including enemy accumulation, traits and abundance of the invasive alien plant itself, and evolutionary processes. For example, investigating recently established and up to 140 year old populations of the invasive *Alliaria petiolata* (garlic mustard), Lankau et al. (2009) revealed that allelopathic compound production declined with time since invasion, presumably due to changes in coevolutionary relationships with microbes and plant competitors (Lankau 2012).

Indeed, the novel weapons hypothesis has been proposed as one of the mechanisms to explain the success of invasive species (Callaway and Ridenour 2004). It suggests that allelopathic biochemical compounds of invasive plants might affect evolutionarily naïve species more strongly compared to plant species in their native range (Callaway et al. 2008; Inderjit et al. 2011). Allelopathy is an interference competition strategy defined as the release of biochemicals by a plant to reduce the establishment and growth of other plants nearby (Inderjit et al. 2011). The allelopathic impact is mediated by abiotic conditions, soil chemistry and texture, the exuded dose, other organisms (e.g., competitors, consumers and inducers), and potentially, lead to evolutionary and adaptive changes across space and time, as showcased by *A. petiolata* and others (Strayer et al. 2006; Lankau et al. 2009; Inderjit et al. 2011; Iacarella et al. 2015; Shannon-Firestone and Firestone 2015; Gruntman et al. 2017). While soil microbes readily degrade allelopathic compounds (e.g., Kaur et al. 2009), degradation may be impeded for invasive species if the microbial communities are evolutionarily naïve (Inderjit and van der Putten 2010; Li et al. 2017). Yet, frequent demonstrations of microbial communities alleviating or eliminating allelopathic effects of invasive plants suggest that soil communities can adapt or evolve to degrade the allelopathic compounds (Lankau 2010; Zhu et al. 2011). With exposure and time since invasion, a higher degradation ability may accumulate (Li et al. 2015, 2017).

Besides microbial legacies that plants leave in the soil (Hannula et al. 2021), allelopathic compounds of invasive plants can affect other plant species directly and indirectly via legacies of changes in microbial communities such as reduced fungal richness or altered community composition (Cipollini et al. 2012; Grove et al. 2012; Souza-Alonso et al. 2015; Roche et al. 2020; Zhang et al. 2021a). While there has been mixed evidence for the persistence of allelopathic chemicals per se after the removal of invasive plant species (Wardle et al. 1998; Inderjit and Callaway 2003; Tian et al. 2007; Del Fabbro and Prati 2015), changes in microbial communities can accumulate (Li et al. 2017; Zhang et al. 2020) and persist longer as legacy effects (Kulmatiski and Beard 2011; Grove et al. 2012, 2017a; Del Fabbro and Prati 2015). Legacy effects via changes in fungal communities have been shown to particularly affect succeeding vegetation (Grove et al. 2012, 2017b; Heinen et al. 2020; Roche et al. 2020), while bacterial legacies might fade quicker (Hannula et al. 2021). Soil legacy effects can also be positive for succeeding plants. For

example, Del Fabbro and Prati (2015) reported that when native plants grew in mixtures with invasive species, soil legacies appeared to alleviate immediate negative allelopathic interference of the invasive species. However, no soil legacy effects were observed when native plants grew in monocultures on soils conditioned by invasive plants. These authors speculated that different biochemicals could be involved over time and microbial communities could adapt to degrade these biochemicals, or the degrading biochemical could fertilise the soil (Del Fabbro and Prati 2015).

Lupinus polyphyllus (henceforth lupine), the model species studied here, exudes numerous allelopathic alkaloids such as lupanine, sparteine, and 4-hydroxylupanine (Wink et al. 1983). These quinolizidine alkaloids can inhibit seed germination and seedling growth (Wink 1983; Muzquiz et al. 1994; Loydi et al. 2015; Lyytinen and Lindström 2019), and the growth of bacteria and fungi (Wink 1984). As lupine is often considered a non-mycorrhizal plant (Oba et al. 2001), it could potentially inhibit mycorrhizal symbiosis, and the growth of plants dependent on mycorrhiza (sensu Grove et al. 2012; Roche et al. 2020). In Finland, where this study was conducted, the invasive lupine has no native congeners from the *Lupinus* family, and thus, the soil microbial communities could be evolutionarily naïve. However, other legume species do occur and lupine was introduced and spread to natural habitats more than a century ago (Lampinen and Lahti 2019). In the study region, it has been recorded from 1936 (Erkamo 2021) but remained rare until the 1980s (Hovi Antti, unpublished results). Now, it is a common and widespread plant in southern Finland. Thus, if the regional scale is relevant, lupine could have evolved during the invasion process (sensu Lankau 2012), or its soil microbial communities could have evolved to degrade its biochemicals (sensu Li et al. 2015). Otherwise, degradation capacity could accumulate with time since invasion at the population scale. Lupine forms nodules in symbiosis with bacteria that fix nitrogen (Ryan-Salter et al. 2014; Kalske et al. 2022a), but besides soil nitrogen levels, *Lupinus* species can enhance the soil carbon and organic matter content (Evans et al. 1987; Halvorson et al. 1991; Titus 2009). Thus, *Lupinus* species can have both facilitating and inhibiting effects on co-occurring plants that may change with community age (Morris and Wood 1989).

To examine complex interactions, and in particular, how the allelopathic effects of invasive lupine could change with local invasion history, or with time since invasion, we grew native, mycorrhizal *Leucanthemum vulgare* (ox-eyed daisy) plants with and without lupine in a greenhouse experiment, where we utilized field soils of varying exposure time to lupine (ca. ≤ 5, $10, \geq 15$ years since local lupine invasion, and controls with no exposure). We added activated carbon (AC) to half of the pots to neutralise potential allelopathic biochemicals. We investigated 1) whether allelopathy affects the growth of the native plant when grown in the presence or absence of lupine, and 2) the effect of soil legacies (i.e., exposure time to lupine) on this potential allelopathic impact. We hypothesised that lupine would reduce the growth of *L. vulgare* (henceforth daisy) more without AC compared to soils with AC (i.e., direct allelopathy), but this difference would be reduced in soils with longer exposure to lupine, for example, due to the adaptation of microbial communities to degrade its allelopathic biochemicals. Alternatively, if potential changes in microbial communities are detrimental to daisy, it would grow worse in soils with longer exposure to lupine. We additionally controlled whether AC or different times since lupine invasion would affect lupine itself, since, for example, alien species can accumulate belowground enemies with local residence time (e.g., Stricker et al. 2016).

Material and methods

Study species

Lupinus polyphyllus Lindl. (Fabaceae) is a perennial herb originating from western North America but has become regionally invasive in temperate-humid climates worldwide (Eckstein et al. 2023). In its invasive range, lupine can grow 50–120 cm tall (Mossberg et al. 2005). It can dominate plant communities with a cover of over 60–90% and may cause significant changes in species richness and the composition of invaded plant communities and associated arthropod abundances (Otte and Maul 2005; Valtonen et al. 2006; Ramula and Pihlaja 2012; Ramula and Sorvari 2017; Prass et al. 2022). In the introduced range, lupine grows in various open environments, from dry ruderal habitats to moist meadows and open

pine forests but thrives especially along road verges in Fennoscandia (Mossberg et al. 2005; Robson et al. 2008; Fremstad 2010; Ramula and Pihlaja 2012; Eckstein et al. 2023).

Leucanthemum vulgare Lam. (Asteraceae) is a perennial herb native to Europe, but has become invasive in North America, Australia and parts of Asia where it can form dense stands (Clements et al. 2004; McDougall et al. 2018; Stutz et al. 2018). Daisy can grow 20–70 cm tall and thrives in a wide variety of open habitats and soils (incl., nutrient levels), although it often grows better on nutrient-poor soils as it does not tolerate strong interspecific competition of dense vegetation (Clements et al. 2004 and references therein). Daisy forms vesicular–arbuscular mycorrhizal (VAM) and AM symbioses (Wang and Qiu 2006; Noori et al. 2014). We chose daisy to investigate allelopathic effects because it often occurs in the same habitats with lupine and is not particularly sensitive to changes in soil nutrient levels (i.e., we can test microbial legacy effects irrespective of potentially higher nitrogen levels due to lupine). Moreover, to our knowledge, no allelopathic effects have been associated with daisy (Rowland 2012), although it has been suggested to have some allelopathy potential (Beres and Kazinczi 2000; Stellick et al. 2020).

Experimental setup

This study was performed in a greenhouse at Viikki campus, University of Helsinki, Finland, using potted plants. We utilised field soils rather than short-term conditioning or microbial inoculation into purchased or sterilised soils because field soils host natural microbial communities and abiotic soil qualities that can influence allelopathic effects and coexistence with other species (Brinkman et al. 2010; Inderjit et al. 2011; Kalske et al. 2022a). In late November 2018, we collected soil from 16 gravelly or sandy grassland sites around the city of Lahti (60°59′N, 25°39′E), southern Finland (see Prass et al. 2022 for additional details) because lupine and daisy frequently occur in these soils in Finland. At each site, we collected six 7 L soil samples: three from lupine-invaded areas and three from uninvaded control areas (i.e., no lupine plants) at least

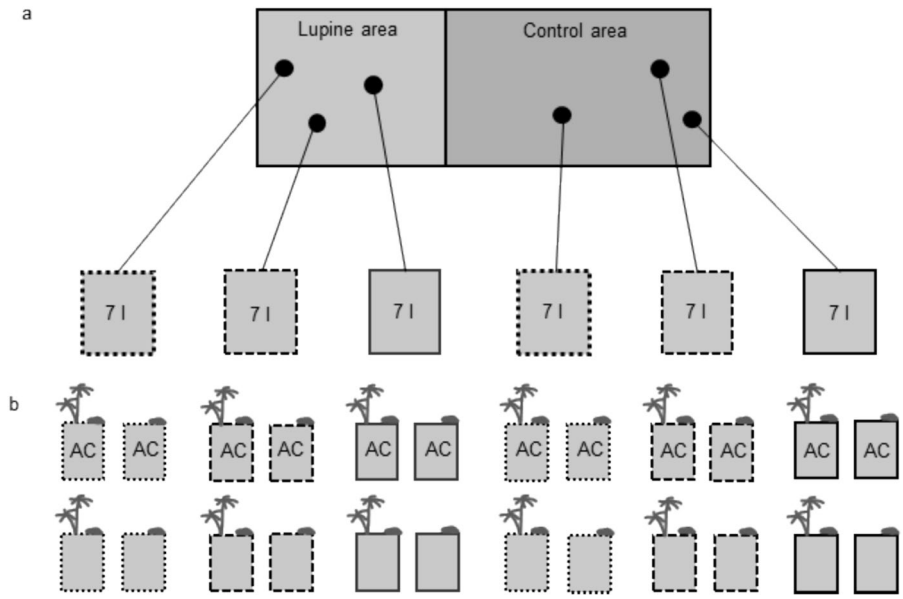

Fig. 1 Setup of the greenhouse experiment. **a** Soil for the experiment was collected from three random locations in lupine-invaded areas and in control areas without lupine individuals at 16 paired sites (i.e., all sites contained both areas). **b** Each sample was then divided between four treatments: 1) *Leucanthemum vulgare* (daisy) with *Lupinus polyphyllus* (lupine) (competition) and activated carbon (AC), 2) daisy without lupine but with AC, 3) daisy with lupine and without AC, and 4) daisy without AC and lupine. In the figure, lupine is denoted as a tall plant and daisy as a short plant. The setup totalled in 384 pots

10 m from the invaded area (Fig. 1a), because we could not observe any lupine individuals at such distance, and past existence was unlikely given the disturbance history of the sites as explained below. We also chose the 10 m distance to ensure that soil conditions are as similar as possible per site. In the invaded area, soil was taken from the rhizosphere of adult lupine plants to obtain lupine-affected soil, whereas soil in the control areas was collected in similar volume and from the same depth at random locations under various plant species. Rocks and plant roots were discarded. Aside from lupine presence and slightly reduced plant species richness at lupine-invaded sites, plant communities in the study sites were generally similar at lupine-invaded and uninvaded areas and did not differ in relation to time since lupine invasion (see Prass et al. 2022). Before collecting another soil sample, shovels were washed with tap water and sterilised by sprinkling pure ethanol onto the wet shovels. The soil samples were then stored individually in plastic bags in an outdoor storage room at approximately 5 °C until being divided into pots in a greenhouse.

To examine the influence of time since invasion on the competitive impacts of lupine, the field sites for soil collection were identified based on their estimated lupine invasion age, besides soil type. After mapping lupine occurrences and their soils in the Lahti area, we used a combination of site visits, yearly orthoimages (Lahti city map portal 2018) and information from residents to ascertain when lupine established. We were able to identify a major disturbance resulting from, e.g., demolition or construction activities, affecting the entire site. Although we cannot exclude the possibility of lupine occurring in the area prior to disturbance, we believe that our age estimates are reasonably accurate. Due to known lupine populations, it was possible to track their development from orthoimages, where they were visibly distinct from other vegetation. In addition, we interviewed residents and could often confirm our estimated lupine invasion time. Based on our findings from orthoimages and interviews, lupine was introduced either intentionally or accidentally, presumably with machinery. As a limitation to our approach, we could not find lupine populations that had gone locally extinct. Likewise, we cannot exclude the possibility of extinct microsites or individuals having occurred outside of the main lupine invasion. However, we did not notice such individuals 10 m away from the invasion front.

We classified the soil collection sites into 3 groups based on the estimated time since lupine establishment at the invaded area of each site (see Prass et al. 2022), which was associated with disturbance in both lupine-invaded and control areas. Invaded areas were exposed to lupine for approximately 5 years, 10 years or 15 years, i.e., young (n = 6), intermediate (n = 7) and old (n = 3) invasions, respectively (Fig. 1). We were able to locate only three sites with old lupine invasions that had gravelly or sandy soils. At each site, there were no obvious differences in soil conditions between the control and invaded areas, both were disturbed at the same time, and we assume that the location of lupine invasion is random. However, to consider any possible differences in soils between the control and invaded areas, we analysed several soil variables (see Soil analysis below).

In the greenhouse, any remaining stones ($\varnothing \geq 2$ cm) and larger plant material were removed, and each soil sample was mixed well with a garden spade. To avoid homogenizing the microbial communities of the soil samples, we used gloves and sterilised all contact surfaces with ethanol (70%) after first washing with tap water and drying these. Each soil sample was divided into four 1.5 L pots ($\varnothing = 13$ cm), representing the four treatments (Fig. 1b): 1) daisy with lupine (competition) and activated carbon (AC), 2) daisy without lupine but with AC, 3) daisy with lupine and without AC, and 4) daisy without AC and lupine. As a result, the total number of pots was 384 (16 sites × 4 treatments × 3 soil histories × 2 areas (lupine-invaded, control)). For treatments 1 and 2, we mixed 20 ml of AC per litre of soil to neutralize allelopathic chemicals in the soil and detangle an allelopathic effect from other potential effects of lupine. The concentration of AC we used (2% volume) has also been used in previous experiments on allelopathy (e.g., Inderjit and Callaway 2003; Lau et al. 2008; Del Fabbro and Prati 2015). As AC, we used a commercially available water purifier grade Aktivt Kol (0.35—1.18 mm) from Clean Carbon (Sweden). AC itself can produce artifacts (Zhang et al. 2021b), but a full-factorial setting allowed us to distinguish the potential effects of AC per se on the native plant. Thus, to maintain the natural microbial communities, we did not fertilise the soils even though AC may improve nutrient availability (Lau et al. 2008). During soil preparation, soils

in the pots were air dried until seeds were added to the pots.

We added 5 lupine seeds to half of the pots (i.e., treatments 1, 3) in March (week 10 of year 2019) (Fig. 1b). The seeds originated from a single lupine stand not used in this study. These seeds were surface sterilised by submerging into ethanol (70%) for 5 s and then rinsed with ample clean water. To improve germination, the lupine seeds were subsequently scarred by carefully chipping the seed coat with scissors without harming the cotyledons. Lupine seeds were germinated in the pots under a plastic film for 2 weeks and grown until 1 month old (week 15). We then chose one of these seedlings and planted it in the centre of the pot while the rest were discarded. The initial set-up with similarly aged daisy plants failed, as the purchased daisy seeds failed to germinate in the pots. Three months later, in June (week 23), we planted 1 month old daisy seedlings next to the 3 month old lupine plants. The daisy seedlings were purchased from Terolan Taimitarha (Tuulos, Finland) where they were grown in a potting soil, which we carefully rinsed off with tap water before planting. The daisy seedlings were always planted to the same location irrespective of treatment: 2.5 cm from the edge and centre of the pot. The pots were placed randomly on four tables with abundant drainage gutters, and the location of pots was changed monthly to avoid potential differences in growing conditions. The pots were weeded and watered regularly. The distance between individual pots was ca. 15 cm. We watered the pots from above delicately to reduce potential cross-contamination between individual pots. All the equipment, gloves and tables were cleaned with ethanol (70%) as needed (e.g., when relocating the pots or weeding).

We measured the diameters of lupine and daisy plants from the top view in the beginning of the experiment (i.e., week 23 of year 2019), and then after one and two months (i.e., at finishing). Since lupine leaves can be distributed unevenly and far from the centre, we also measured plant height and number of leaves for lupine, but these correlated with plant diameter and were not used in the analyses ($r = 0.6$ for both height and leaf number with plant diameter). To measure the final biomass of plants, we delicately extracted plants from the pots at the end of the experiment in July (i.e., week 31) and washed the roots thoroughly with tap water to remove any traces of soil. The plants were then dried in an oven at 70 °C for 48 h, and weighted for total, above- and belowground biomass. Due to strong correlations between the three biomass values ($r > 0.7$ for all cases), we only analysed total biomass. Similarly, lupine diameter correlated with its total biomass ($r = 0.7$), and we thus analysed only the more precise total biomass for lupine, and not diameter.

Soil analysis

Despite selecting sandy or gravelly sites, soil structure varied within and between sites. To assess variation, we analyzed the collected soil samples for ammonium (NH_4), nitrate (NO_3), phosphate (PO_4) and organic matter (OM) content as well as for soil pH and moisture. The three soil samples per area of a site were pooled and mixed (Fig. 1a), stones ($\varnothing = 1.5$ cm) and plant material were removed, and this resulting pooled soil sample was analyzed as follows. We extracted the water-soluble soil nutrients with distilled water (standard SFS-EN 13652) and measured using a QuickChem Lachat 8000 flow injection analysis system (LACHAT Instruments Inc., Loveland, CO, USA) according to standards 10-107-06-1-F for ammonium, HPLC K101-NO3-140,407 for nitrate and 10-115-01-1-Q for phosphate. Soil moisture and OM (i.e., loss on ignition) content were measured using air-dried soil, which was then dried at 105 ℃ for 2 h and subsequently again for 2 h at 550 ℃, respectively (standard SFS3008). Soil pH was measured from a suspension of air-dried soil and distilled water (1:5 v/v) (standard ISO 10390).

Overall, environmental characteristics of soils were similar (Supplementary Information SI1). In relation to invaded and control areas, there were no meaningful differences in soil pH or NO_3 levels, but the soil from control areas had a slightly higher moisture, OM, NH_4 and PO_4 content than the lupine-invaded areas. However, the comparatively higher means of control areas were influenced by single values. Regarding invasion age, there were no clear differences in soil variables, only the mean OM content and mean moisture were slightly higher in old lupine invasions compared to young and intermediate invasions (i.e., 6.6% and ca. 4%, respectively; Supplementary Information SI1).

Data analysis

To investigate the effects of lupine allelopathy and its soil legacies on the growth of daisy, we utilized mixed-effects models (lme4 package; Bates et al. 2015) in R (R Core Team 2024). We also tested for legacy effects and AC addition on the growth of lupine itself. Prior to analysis, we followed the protocol proposed by Zuur et al. (2010) to explore data for outliers, homogeneity, normality, zeros, collinearity, relationships, interactions and independence. Soil variables were not included in the models, as we employed a full-factorial design in the greenhouse, and soil conditions were similar. For both daisy and lupine, we analysed total biomass (see above). For daisy, we additionally calculated a relative growth rate (RGR) for the first and second month separately as in the equation below, where D_0 is the initial plant diameter and D_1 is daisy diameter one month later, and $t_1 - t_0$ refers to time between the two measurements (here one month). RGR was not correlated with other daisy measurements.

$$RGR = \frac{1}{D_0} \times \frac{(D_1 - D_0)}{(t_1 - t_0)}$$

For daisy response variables, we analysed total biomass (Gamma distribution) with a log-link generalised linear mixed-effect model (GLMM, lme4::glmer), and RGR (Gaussian distribution) with a linear mixed-effect model (LMM, lme4::lmer4). Based on our hypotheses, both models included the following fixed explanatory variables: competition (a factor with 2 levels: with and without lupine), AC addition (yes, no), area (invaded, control), age (young, intermediate, old), and all possible interactions among the fixed variables. The models also included a nested random factor in which soil samples were nested within area, which was further nested within site (i.e., site/area/soil sample) to account for the non-independence of multiple observations at each level (i.e., four treatments per soil sample, three soil samples per area, and two areas per site).

To explore potential effects of AC and soil legacies on lupine itself, the total biomass of lupine (Gaussian distribution) was analysed with a linear mixed-effect model. Like models for daisy, it included a nested random factor (i.e., site/area/soil sample), and the same fixed explanatory variables apart from competition: AC addition (yes, no), area (invaded, control), age (young, intermediate, old), and all possible interactions among the fixed variables. Model assumptions were verified for all mixed-effects models by plotting residuals versus fitted values. Following the analyses, significance of the fixed variables was determined using a Wald chi-square test for GLMMs (car::Anova) and an F-test for LMMs (lmerTest::anova).

Results

By the end of the experiment, total biomass of daisy was significantly reduced when grown in competition with lupine. While AC addition (an agent to neutralize allelopathic effects) generally increased biomass, this occurred irrespective of the presence or absence of lupine (Table 1 and Fig. 2a, see also Supplementary Information SI2 for full GLMM results). Without competition, daisy grew larger in previously lupine-invaded soils compared to uninvaded control soils, as indicated by a significant competition × area interaction (Table 1and Fig. 2a). Age of lupine invasion at the soil collection sites did not explain daisy biomass, neither individually nor in relation to other variables (Table 1).

Competition with lupine was associated with reduced relative growth rate of daisy, particularly in the first month and to a lesser extent in the second month (Table 1 and Figs. 2b, c). In the first month, the growth of daisy was more than two times slower next to lupine than when grown alone (Fig. 2b). In the second month, AC addition significantly increased the growth of daisy when it grew alone, whereas the opposite tended to be true when it grew together with lupine (Table 1 and Fig. 2c). However, this effect of AC was not related to soil origin (lupine-invaded vs. control). In general, site age did not affect the growth of daisy (Table 1). However, for growth in the second month, there was a significant competition × site age × area interaction (Table 1). Without competition, daisy grew faster in old soils collected from control areas than in old soils collected from lupine-invaded areas (Fig. 2c). Yet, the opposite pattern occurred in the competition setting with the same soils – daisy grew faster next to lupine in old invaded soils than in old control soils (Fig. 2c). The AC treatment was insignificant in this interaction, albeit, when grown alone, daisy grew comparatively slower without AC

Table 1 Results from the mixed-effect models of *Leucanthemum vulgare* (daisy) and *Lupinus polyphyllus* (lupine) to examine the effects of lupine's allelopathy (AC addition) and soil legacies on the growth of both species

Response variable	Explanatory variable	$Statistic_{df,ddf}$	*p*-value
Leucanthemum vulgare			
Total biomass	Competition with lupine (yes/no)	617.20_{1}	**< 0.001**
	AC addition (yes/no)	5.77_{1}	**0.016**
	Site age (young, intermediate, old)	0.83_{2}	0.659
	Area (invaded, uninvaded)	0.40_{1}	0.526
	Competition × AC addition	0.68_{1}	0.408
	Competition × site age	0.49_{2}	0.782
	Competition × area	10.07_{1}	**0.001**
	AC addition × site age	0.08_{2}	0.957
	AC addition × area	2.73_{1}	0.098
	Site age × area	0.65_{2}	0.720
	Competition × AC addition × site age	0.12_{2}	0.942
	Competition × AC addition × area	0.25_{1}	0.614
	Competition × site age × area	0.01_{2}	0.996
	AC addition × site age × area	0.84_{2}	0.656
	Competition × AC addition × site age × area	4.71_{2}	0.095
Relative growth rate, 1st month	Competition	$245.99_{1, 267}$	**< 0.001**
	AC addition	$0.02_{1, 267}$	0.876
	Site age	$0.48_{2, 12}$	0.631
	Area	$0.56_{1, 13}$	0.468
	Competition × AC addition	$0.50_{1, 267}$	0.478
	Competition × site age	$2.31_{2, 267}$	0.101
	Competition × area	$1.11_{1, 267}$	0.292
	AC addition × site age	$1.37_{2, 267}$	0.255
	AC addition × area	$0.12_{1, 267}$	0.730
	Site age × Area	$0.32_{2, 13}$	0.731
	Competition × AC addition × site age	$1.11_{2, 267}$	0.329
	Competition × AC addition × area	$1.84_{1, 267}$	0.177
	Competition × site age × area	$0.39_{2, 267}$	0.678
	AC addition × site age × area	$0.45_{2, 267}$	0.640
	Competition × AC addition × site age × area	$2.56_{2, 267}$	0.079

Table 1 (continued)

Response variable	Explanatory variable	$Statistic_{df,ddf}$	*p*-value
Relative growth rate, 2nd month	Competition	$3.84_{1, 330}$	0.051
	AC addition	$1.08_{1, 330}$	0.299
	Site age	$0.06_{2, 26}$	0.941
	Area	$0.07_{1, 26}$	0.794
	Competition × AC addition	$8.57_{1,330}$	**0.003**
	Competition × site age	$2.73_{2, 330}$	0.067
	Competition × area	$2.44_{1,330}$	0.120
	AC addition × site age	$2.35_{2, 330}$	0.096
	AC addition × area	$0.58_{1, 330}$	0.445
	Site age × area	$0.57_{2, 26}$	0.568
	Competition × AC addition × site age	$0.22_{2, 330}$	0.799
	Competition × AC addition × area	$1.24_{1, 330}$	0.267
	Competition × site age × area	$3.20_{2, 330}$	**0.042**
	AC addition × site age × area	$0.37_{2, 330}$	0.690
	Competition × AC addition × site age × area	$0.55_{2, 330}$	0.578
Lupinus polyphyllus			
Total biomass	AC addition	$4.54_{1, 90}$	**0.035**
	Site age	$1.53_{2, 13}$	0.252
	Area	$0.01_{1, 13}$	0.925
	AC addition × site age	$5.77_{2, 90}$	**0.004**
	AC addition × area	$0.31_{1, 90}$	0.578
	Site age × area	$0.15_{2, 13}$	0.859
	AC addition × site age × area	$0.16_{2, 90}$	0.850

Statistic refers to $\chi 2$ (daisy biomass) or F-value (all other), and df and ddf denote the degrees of freedom in the numerator and denominator, respectively, when applicable. Age represents lupine invasion age at the soil collection site. Bold values represent statistical significance at p-value < 0.05. See Supplementary Information SI2 for full GLMM results

in the second month in soils collected from control areas and old sites than in the same soil with AC (Fig. 2c). However, variation was high for old soils due to small sample size (Fig. 2).

The final total biomass of lupine was not significantly related to area nor invasion age (Table 1). Like daisy, lupine generally grew larger with AC addition and particularly so when grown in old soils (Table 1 and Fig. 2d).

Discussion

Using soils from field sites of varying lupine exposure time, this greenhouse experiment examined legacy effects on coexistence mechanisms; whether allelopathic competition of lupine changes with time since invasion. Lupine reduced the size of daisy, a competing native plant, by significantly impeding its growth particularly in the first month of the experiment. In contrast to our hypothesis, this negative effect on daisy was not ameliorated by activated carbon, which implies that lupine was not allelopathic to daisy in our experiment. Furthermore, the suppressive effect of lupine on daisy did not appear to be affected by invasion history of the soil source (lupine-invaded, uninvaded control) nor time since invasion at the lupine-invaded sites. Also, lupine itself was not significantly affected by soils of varying lupine exposure times. Thereby, our

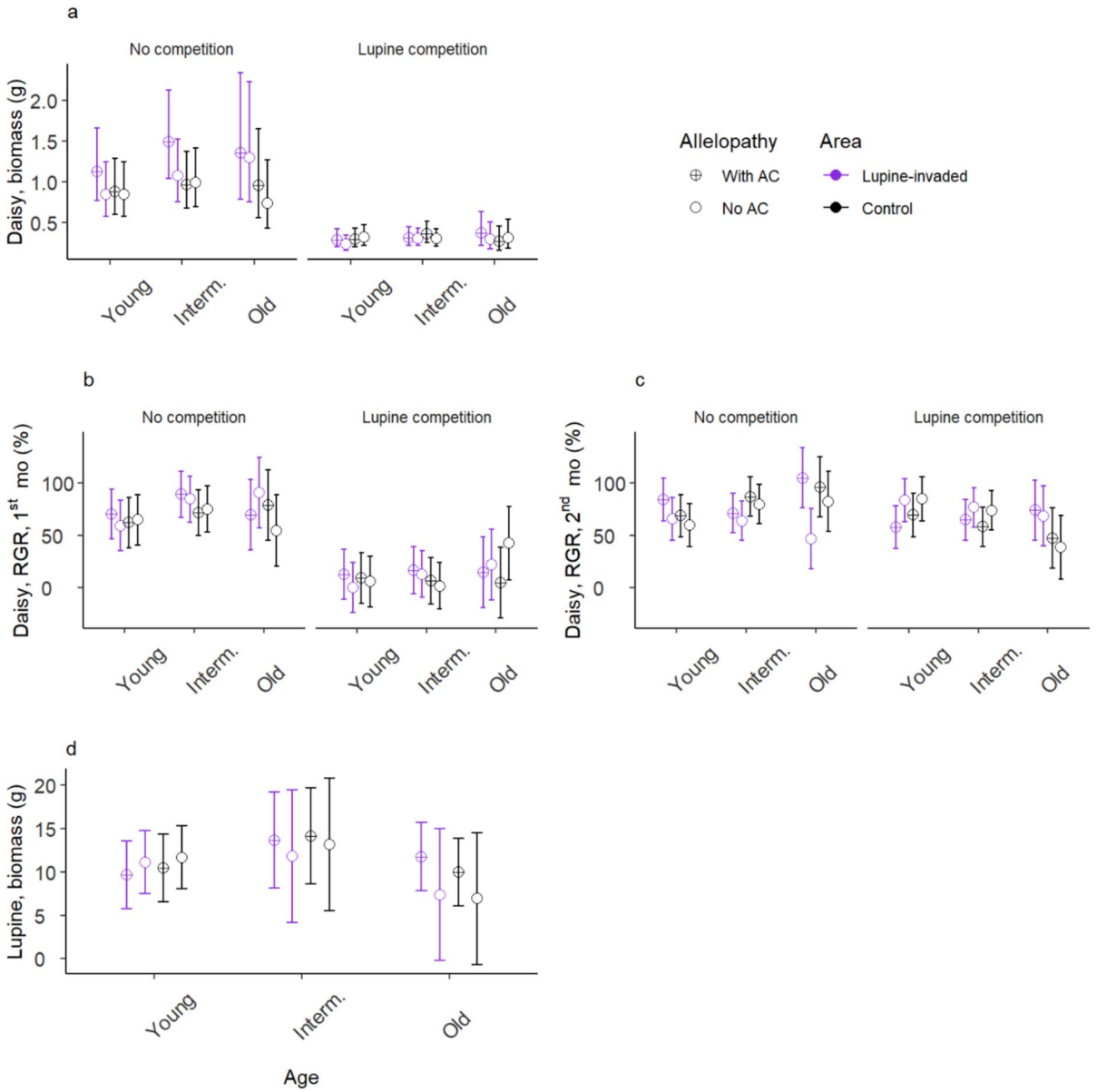

Fig. 2 Predicted plant size (mean ± 95% CI based on fixed explanatory variables only) for *Leucanthemum vulgare* (daisy) and *Lupinus polyphyllus* (lupine) based on mixed-effects models (Supplementary Information SI2): **a** Total biomass of daisy, **b** Relative growth rate (RGR) of daisy diameter in the first month, **c** RGR of daisy in the second month, and **d** Total biomass of lupine. All daisy figures (**a**-**c**) show conditions when daisy is grown in competition with lupine or on its own, and with or without activated carbon (AC) addition (i.e., a proxy to allelopathy effect). The plants were grown in field soils that originated from sites with young, intermediate or old lupine invasions (i.e., ca 5, 10 and 15 years, respectively), and either from invaded or control areas without lupine

results show no temporal changes in the ecological impacts of the invasive lupine on daisy or on its own performance. Albeit contrary to our hypotheses, this is in line with our previous results from a field study of lupine (Prass et al. 2022), where time since invasion on a population scale was not associated with vascular plant species richness nor lupine cover.

The apparent lack of allelopathic effects and associated soil legacy effects on daisy might be explained by differences in study designs between the present and previous studies. The allelopathy of lupine has been mostly concluded from experiments with lupine leachates and seed germination or the establishment of young seedlings when allelopathy is most pronounced (Eckstein et al. 2023 and references therein). For example, Loydi et al. (2015) found that in several plant species, including lupine, such negative effects largely ceased after delaying germination. In general, allelopathic interference is weaker for experiments using soils compared to leachates or plant residues, and with longer experimental durations (a meta-analysis by Zhang et al. 2021b) when negative effects on the performance of a competing plant are likely dominated by other competition mechanisms than allelopathic interference. Here, we found that lupine had no allelopathic effects on 1– 3 month old daisies in a full-factorial set-up that deployed activated carbon to neutralise the allelopathic compounds (if any).

Another explanation for no effect could be that evolutionary processes may have been involved, given the high intra- and interpopulation variation in genetic diversity of lupines (e.g., Galkina et al. 2025). Indeed, Kalske et al. (2022b) found that lupine alkaloid diversity was lower in Finland than in native populations, indicating a potential trait evolution in response to loss of native enemies and changes in resource availability. As a limitation to this study, we did not measure the content of allelopathic compounds per se, nor their potential evolutionary changes. Previously, Lyytinen and Lindström (2019) suggested that allelopathy of lupine is weak. In their common garden experiment with potted plants in Finland, the final biomass of cow parsley (*Anthriscus sylvestris*) appeared to benefit from AC addition during the first growing season, but not in the second growing season. Importantly, that benefit from AC was similar to their fertiliser treatment, and AC increased the final biomass of cow parsley irrespective of the presence of lupine. Wurst et al. (2010) also report weak allelopathy in Germany, lupine suppressed the adjacent ribwort plantain (*Plantago lanceolata*) only in the first days of seedling establishment. In our experiment, we used daisy seedlings that were 1-month old at the beginning of the experiment. They might have been less sensitive to allelopathy compared to younger seedlings, but they were probably exposed to more natural concentrations of allelopathic compounds alongside with natural levels of soil microbes and abiotic conditions compared to leachate experiments, allowing a more realistic insight into coexistence.

While we did not find evident legacy effects from field soils, lupine did evidently reduce daisy growth in the first month but not in the second. The fact that AC did not alleviate the suppression of daisy growth suggests a lack of evident allelopathic interference to these 1–3 month old seedlings. Instead, growth was likely suppressed because of resource competition. We conclude that an allelopathic effect of Finnish lupine on daisy was negligible here with natural soils and their microbial communities that could have been able to degrade allelopathic compounds. However, we did not study the microbial communities explicitly. Due to the limitations of this study, we cannot assess whether allelopathy may be pronounced in different settings (e.g., microbial or evolutionary), or exist during seed germination or at earlier stages of seedling establishment and subsequently influence plant communities through differences in plant sensitivity to allelopathic compounds (Zhang et al. 2021b), but these life stages were not considered in our study.

We cannot entirely rule out the possibility that AC was ineffective at binding lupine allelochemicals, but we find it unlikely because we utilised a full-factorial set-up. If AC was ineffective in binding allelopathic compounds, combinations with lupine plants or with lupine-exposed soils would have been different in comparison to those without exposure. Secondly, AC has previously been demonstrated to mitigate growth suppression by lupine (Loydi et al. 2015; Lyytinen and Lindström 2019). However, the relationship between AC addition and lupine competition or soils (area) were all insignificant in our study. Only during the second month of daisy growth, AC significantly interacted with lupine competition—daisy grew slower with than without AC adjacent to lupine. This result likely arose from lupine growing larger with AC addition and hence suppressing the growth of daisy more than without AC. It appears that AC itself caused artefacts—albeit not consistently, it increased the biomass of both lupine and daisy irrespective of competition (Fig. 2a, d). Also, Lyytinen and Lindström (2019) showed that AC improved growth of both lupine and cow parsley (see above). Inconsistency in AC effects here likely resulted from small variations in the quality of natural soils used, since

AC particles interact with bulk density and porosity of sandy soils (Głąb et al. 2016). In addition, because we were only able to include three true replicates for old lupine invasions, interpretation for the significant AC and age interaction of lupine biomass is limited.

In line with the lack of evidence for allelopathic effects here, our results do not show clear soil legacy effects or more specifically, time since invasion effects. Previously, numerous allelopathic invasive plants have been reported to cause significant microbial changes across various soils (e.g., Grove et al. 2012, 2017b; Roche et al. 2020; Unger et al. 2022). Furthermore, allelopathic plants can have a disproportionately stronger negative impact on AM fungi and mycorrhizal plants, as showcased for invasive garlic mustard in forest plots (Roche et al. 2020): after weeding the garlic mustard, it took 4–6 years to observe clear changes in the plant communities, which they speculated could have corresponded to the recovery time of mycorrhizal fungal communities. However, here, the mycorrhizal daisy grew slightly larger in lupine soils when grown without lupine. In the competition setting, this minor benefit was no longer visible, highlighting the importance of studying legacy effects under more natural conditions. The improved growth of daisy in invaded soils could be linked to lupine potentially increasing nitrogen and phosphorus content of soils (Evans et al. 1987; Titus 2009) or soil differences, but in our soils, lupine had a negligible effect on soil nutrient content and the used soils were comparatively similar in relation to non-invaded and lupine-invaded areas (Prass et al. 2022). Alternatively, potential microbial changes in the soils could have been favourable to daisy when grown alone.

Similar to the daisy, lupine itself appeared to be unaffected by soil invasion history, or exposure time—its final biomass was not significantly related to soil collection area (invaded/uninvaded), age of lupine invasion or their interaction. However, this conclusion must be treated with caution since we only had three sites with 15 year old lupine invasions. Furthermore, due to the length of our experiment, the likelihood of cross-contamination of microbes between pots increased despite careful watering to prevent movement of soil particles and microbes. However, similar to our results, Prass et al. (2022) did not detect changes in lupine covers at differently aged lupine field sites. Ramula (2014) found unexplained differences in lupine population growth rates, with southern and presumably older populations growing faster than central populations in Finland. These results collectively suggest that older lupine-invaded soils per se might not reduce species' performance at individual and population levels. On the contrary, based on lupine invasions (> 10 years old), we have previously observed that lupine tends to benefit from its associated soil microbiota (Kalske et al. 2022a; Ramula et al. 2025).

Soil legacy effects from invasive plants may relate to the temporal stage of invasion and the identity of the study species. Here, the lupine invasions were ca 5 years, 10 years or 15 years old, whereas marked legacy effects have been reported, for example, by Grove et al. (2012, 2017b) from sites with 30 year old dense *Cytisus scoparius* (Scotch broom) invasions, or from at least 20 year old *A. petiolata* (garlic mustard) invasions (Roche et al. 2020). When studying over 10 year old lupine invasions in southwestern Finland, Mousavi and Ramula (2024) found some changes in soil bacterial composition, but these effects were inconsistent across study sites. Currently, lupine-induced changes in fungal communities are unknown and could be potentially more significant, as has been suggested by studying plant communities (Heinen et al. 2020; Hannula et al. 2021). Therefore, it would be valuable to explore lupine-associated microbiota (bacteria and fungi) in relation to soil invasion history. While legacy effects have been well established for nitrogen-fixing *C. scoparius*, Grove et al. (2017b) reported that the suppression of ectomycorrhizal fungi was constant in 3–31 year invasions, it did not change with time. In a review, Grove et al. (2017a) concluded a lack of consistent effects of time since invasion on mutualism disruption, or, alternatively, that the soil-based effects of *C. scoparius* develop within just a few years and do not change over time.

Our findings suggest that competitive impacts of the invasive lupine on co-existing plants occur rapidly and can be stable over time. Lupine significantly suppressed the growth of daisy early in the seedling phase, resulting in a reduced final biomass, but we could not detect clear allelopathic effects nor soil legacies on daisy nor on lupine. Here, we utilised field soils that host natural densities of microbes and concentrations of potential allelopathic chemicals, which provides more realistic conditions compared to short-term conditioning

and soil inoculation, or plant leachates (Brinkman et al. 2010). To investigate complex interactions and allelopathy, we acknowledge the fact that both species should be ideally grown without competitors and under intra- and interspecific competition. Furthermore, studying mechanisms explored here would benefit from long-term monitoring of alien species' study systems (e.g., Dostál 2024), or herbarium records and literature to identify older invasions than used here (e.g., Lankau et al. 2009). Yet, our exploratory study results are aligned with finding of no legacy effects of lupine on vascular plant communities under field conditions (Prass et al. 2022) as well as with Ramula and Kalske (2023) who found no antagonistic effects of microbial communities from lupine-invaded soils on lupine's performance in a common garden experiment with autoclaved soils and intact soil inoculums. Our results contribute to the literature reporting no changes in the performance of invasive plant species with invasion history (invaded, uninvaded) and time since local invasion.

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Data availability Datasets generated during the current study are available from the corresponding author on request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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