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Development of negative soil feedback by an invasive plant near the northern limit of its invaded range

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Abstract

Invasions at high latitudes are recognized as an emerging threat to native biodiversity, but non-native plants still are scarce in northern Canada. One factor potentially inhibiting further invasions may be below-ground interactions; in particular, it is unclear whether interactions with soil biota are likely to help or hinder the spread of new species into often challenging northern soils. In the Canadian subarctic, the non-native plant *Linaria vulgaris* has invaded human-disturbed soils in the town of Churchill, Manitoba (58.8°N) but for decades has failed to spread into natural communities. One explanation for this stasis might be greater resistance by soil communities in uninvaded areas relative to areas where this plant is established; however, no local evidence for plant-soil feedbacks exists. In one of the first papers to test the potential role of plant-soil feedbacks in an invasion at high latitudes, we planted *L. vulgaris* in soil serially inoculated with live and sterilized field-collected soil sampled from invaded or uninvaded sites, and measured plant performance (biomass) over three iterations. We also conducted soil chemical analyses to determine whether pH, and carbon, nitrogen, and phosphorous contents differ between invaded and uninvaded areas. There was no initial difference in biomass between inoculation treatments in the first two iterations. However, by iteration 3, we found that sterilization significantly increased *L. vulgaris* biomass in invaded soils, indicating feedback becomes negative in invaded soils compared to uninvaded soils. Soil chemistry did not differ significantly between invaded and uninvaded soils, though there was a tendency for invaded soils to contain more carbon and nitrogen. These results do not support the hypothesis that *L. vulgaris* is absent from uncolonized sites because soil communities resist invasion. Instead, they provide evidence that *L. vulgaris* is inhibited by plant-soil feedbacks in invaded soils, while feedbacks in native-dominated soils are not a barrier to further local spread. Thus, explanations for the restriction of this species must lie elsewhere.

Keywords Non-native species · Subarctic · Plant-soil feedbacks · Churchill · Range edges

Introduction

Interactions between plants and soil can play a significant role in community assembly and the invasion process (Klironomos 2002; Kardol et al. 2006). Plant-soil feedbacks (PSF, or simply "feedbacks") occur when a plant modifies soil biota or soil properties, subsequently affecting itself and other plants in the community (Bever et al. 1997). It has been postulated that plants typically experience negative soil feedback in their native ranges due to the presence of generalist and specialist pathogens and herbivores, resulting in species coexistence (Bever et al. 1997; Callaway et al. 2004b; Kulmatiski et al. 2008; Inderjit & van der Putten 2010; Suding et al. 2013). In contrast, in invaded ranges, non-native plants may encounter novel soil biota, which can influence the ability of these species to invade (Reinhart et al. 2003; Reinhart & Callaway 2006). For instance, the Enemy Release Hypothesis, which proposes that non-native species escape their natural enemies when invading new regions (Elton 1958; Keane & Crawley 2002), has been cited as a possible cause of reduced negative effects of soil biota in invaded areas (Keane & Crawley 2002; Reinhart & Callaway 2006).

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Many studies have documented that highly invasive species experience reduced negative feedback in invaded areas, accumulating pathogens at a slower rate than in their native ranges, and thus, reaching higher densities in the invaded range (Callaway et al. 2004a; Knevel et al. 2004; Reinhart & Callaway 2004; Maron et al. 2014). There is also evidence that they suffer less from negative feedbacks compared to co-occurring natives in the invaded range, resulting in a greater ability to invade (Van Grunsven et al. 2007; Engelkes et al. 2008). However, pathogens or soil-borne antagonists in the invaded range may be able to adapt to non-native species, leading to limited or no release from enemies (Beckstead & Parker 2003). Mutualistic soil biota such as mycorrhizal fungi and symbiotic bacteria in invaded ranges may also be less beneficial (Klironomos 2003; Nuske et al. 2021), countering any benefits of reduced negative feedbacks. As a result, non-native species that are highly invasive at first may also decline in performance over time as pathogens accumulate (Nijjer et al. 2007; Flory et al. 2018), and feedback becomes more negative (Diez et al. 2010). Finally, invaders themselves have been found to change soil properties (Liao et al. 2008; Gibbons et al. 2017; Zhang et al. 2019), potentially affecting both future invasions and the native community (Vitousek et al. 1987; Schittko & Wurst 2014). Thus, below-ground feedbacks during invasions can be variable (Wolfe & Klironomos 2005), and invasion can either be impeded by interactions with soil biota, or facilitated if there is a net reduction of negative soil feedbacks. Understanding the below-ground interactions between plants and soil during invasion can aid in understanding why some plants are invasive in particular habitats while failing to spread into others, and why some plants fail to invade past range edges even given numerous subsequent introductions (Chung 2023).

Polar regions are relatively resistant to invasions (Wasowicz et al. 2020; Guo et al. 2021). Invasions are a leading threat to native biodiversity in temperate regions (Vitousek et al. 1997; Colautti et al. 2006; Ricciardi et al. 2013), but boreal forests are relatively uninvaded, and northern tundra ecosystems are almost free of invasive plants (Alsos et al. 2015; Conservation of Arctic Flora and Fauna & Protection of the Arctic Marine Environment 2017), likely as a result of harsh environmental conditions and limited human access. However, polar invasions are increasingly recognized as an emerging problem likely to worsen as future climate change ameliorates the environment for non-native plants and promotes increased high-latitude travel and trade (Chen 2012; Myers-Smith et al. 2019). Churchill, Manitoba, on the Hudson Bay coast (58.8°; Fig. 1), represents a uniquely suitable site for research into the risk of northern invasions, and specifically into current limits to invasions. Over a hundred non-native plants have been recorded in Churchill in surveys since 1959 (Beckett 1959; Staniforth & Scott 1991; Kent et al. 2018), reflecting its history as a railway link and a grain and shipping port. Some of these plants have persisted for decades in the Churchill townsite and other human-disturbed areas, resulting in a relatively high rate of invasion compared to other North American locations at similar latitudes, but almost none have spread from this reservoir into the boreal forests and tundra ecosystems (Beckett 1959; Kent et al. 2018). This region, therefore, offers the ideal opportunity to investigate factors currently preventing further invasion of non-native species into northern regions.

Linaria vulgaris Mill. (Common Toadflax) is one of the most locally abundant invaders in the Churchill townsite and nearby anthropogenically modified areas. *L. vulgaris* is a conspicuous herbaceous perennial originating from Europe and Asia; in North America, it is a problematic weed and has invaded a wide range of habitats, especially disturbed areas (Saner et al. 1995). Churchill lies at the northern edge of its known central North American range. Despite being locally present since at least 1946 (Beckett 1959) and spreading widely throughout disturbed sites, *L. vulgaris* has failed to colonize natural tundra and boreal forest, even in locations immediately adjacent to existing populations, and instead remains restricted to patchy populations in disturbed areas, roadsides, dumps, and similar sites. Previous surveys have indicated that it consistently fails to produce viable seed (Staniforth & Scott 1991). Instead, populations persist from a deep, creeping root and rhizome system, and likely spread both by clonal growth and as transported root fragments. Local populations are variable year to year, but *L. vulgaris* populations are relatively larger than other non-native species in Churchill, and it has the potential to become a highly invasive species, as has occurred elsewhere (Saner et al. 1995).

The strength and direction of feedback between its root system and the soil microbiota with which it interacts could have implications for the local persistence and spread of *L. vulgaris*. Maron et al. (2014) provided evidence that *L. vulgaris* escapes root pathogens in its invaded range, compared to its native range. If this is true in Churchill, existing populations of *L. vulgaris* in the townsite might have escaped from strong negative feedbacks, improving their persistence. In the absence of pathogens, such populations potentially could further benefit from the cultivation of appropriate mycorrhizal fungi or other soil mutualists, while the absence of these partners could inhibit the colonization of new sites. *L. vulgaris* associates with arbuscular mycorrhizae (Pendleton & Smith 1983) but may be limited in mycorrhizal associations at high latitudes (Gardes & Dahlberg 1996); this could be a serious limitation in often poorly developed or nutrient-poor arctic soils (Read et al. 2004; Hobbie & Hobbie 2006; Iversen et al. 2015). Thus, evidence of relatively stronger negative feedback in uninvaded soils could help explain the slow spread of this species, despite its ability to persist for decades in areas it has already invaded.

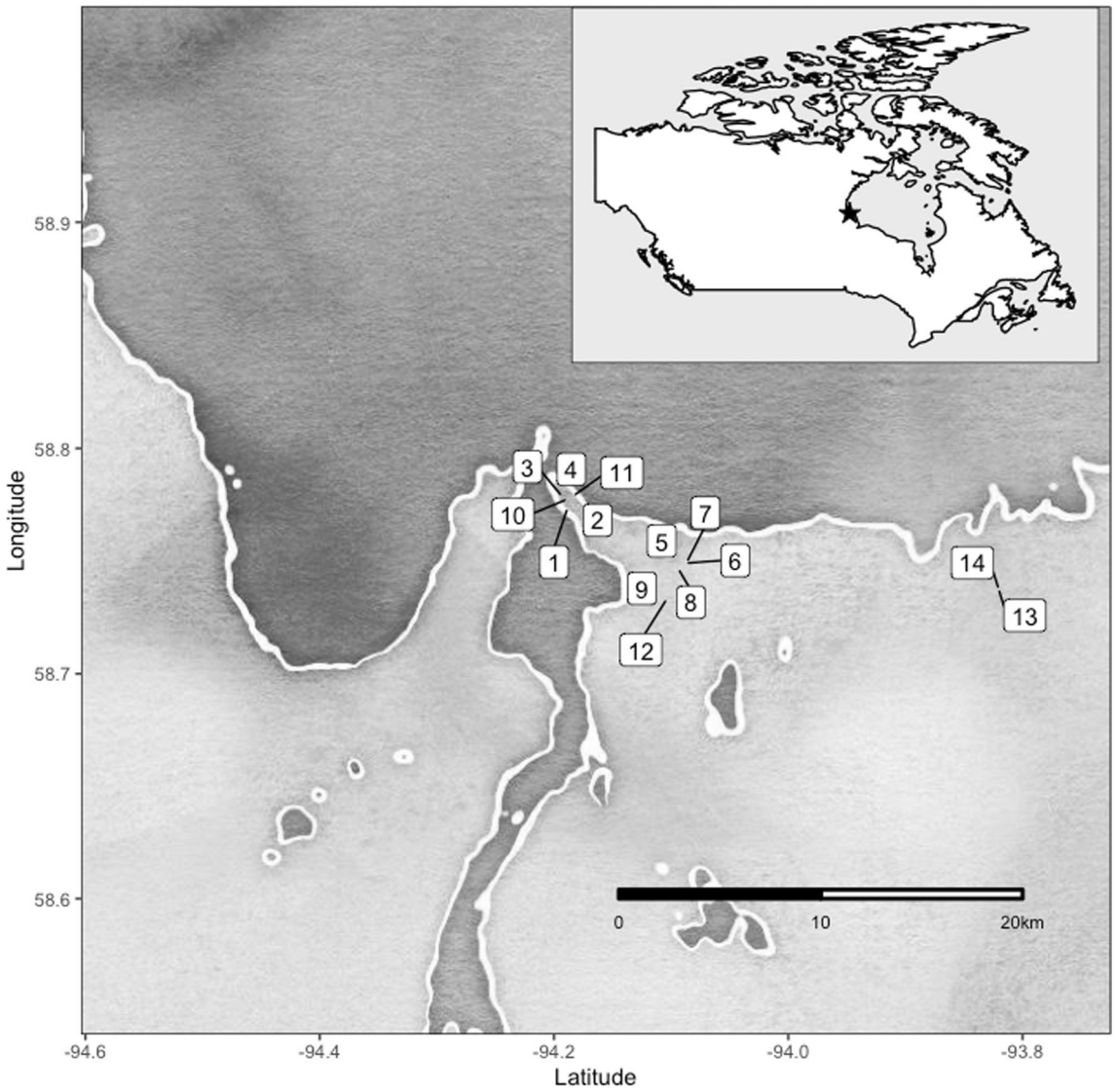

Fig. 1 Main: The 14 sampled sites in Churchill, numbered: 6 in the Churchill townsite, 6 along roadsides east of the Churchill Airport, and 2 on Launch Road next to the Churchill Northern Studies Centre. Inset: Location of Churchill, Manitoba, indicated by the black star. Maps produced using 'ggmap'

Alternatively, if populations of *L. vulgaris* experience stronger negative feedback in invaded soils compared to uninvaded soils, the explanation for its persistence without further spread must lie elsewhere. In this study, we distinguish between these possibilities by using a greenhouse experiment to compare feedbacks in soil inoculated with samples from sites with established populations of *L. vulgaris* versus soil inoculated with samples from nearby uninvaded sites.

Methods

Field sampling

In August 2020, field soil was collected from 14 sites invaded by *L. vulgaris* in the Churchill area (Fig. 1). These sites were spaced at least 10 m apart and were drawn from three general locations: six sites within the Churchill townsite, six sites on roadsides near the airport, and two sites on roadsides near the Churchill Northern

Studies Centre (CNSC). All fourteen sites were anthropogenically disturbed and not natural tundra communities. Overall, samples ranged from an almost purely mineral soil to almost entirely organic.

Within each site, soil was sampled at two plots: the first plot was within a population of *L. vulgaris* (referred to as "invaded"), and the second plot was approximately 2 m away from the population (referred to as "uninvaded"). These uninvaded plots were completely outside of existing *L. vulgaris* populations, beyond the extent of roots (Charlton 1966), and containing no plant or root material. Both paired plots were located in human-modified areas, supported similar vegetation (excluding *L. vulgaris*), and did not obviously differ in abiotic factors such as soil, moisture, temperature, and sunlight exposure. To document sampling sites, GPS locations were recorded, and photographs of the plots were taken. After surface litter was removed, samples were collected using a trowel (depth of 15 cm) and stored separately in Ziploc™ bags and express-mailed back to the University of Toronto Mississauga in an insulated chilled container. Although it is possible cross-contamination occurred during sampling, the amount of soil transferred between samples during collection would have been extremely small compared to the samples collected; as well, if any contamination occurred, it was insufficient to mask the experimental effects detected. Soil was kept in a refrigerator (4 °C) for 3 months prior to use.

Soil chemical analyses

Although our focus was on soil feedbacks, we also checked for any nutrient differences between invaded and uninvaded soils which may also affect plant performance. Each sample of soil was analyzed for total carbon (% dry), nitrogen (% dry), phosphorous (mg/kg), and pH. Chemical analyses of total carbon, nitrogen, and phosphorous were done by the Agriculture and Food Laboratory (AFL) at the University of Guelph. No other macro- or micronutrients were analyzed since local studies have indicated that nitrogen and phosphorous are most limiting in local environments (Henry et al. 1986; Hargreaves et al. 2009; Lang et al. 2021). Three separate pH measurements were taken for each sample using an HQd Portable pH meter (IntelliCAL™ Rugged Field Kit), and these measurements were averaged.

Feedback experiment

Our feedback experiment was conducted in a greenhouse located at the University of Toronto Mississauga in southern Ontario. Because of this, we were unable to simulate all of the factors that affect performance of these plants in the field (Dawson & Schrama 2016); however, a controlled greenhouse setting was necessary as it reduced contamination and environmental noise. Spring-like conditions were maintained for the duration of the experiment (60% humidity, 14-h daily lighting, 17 °C:10 °C day:night temperature cycle). Since *L. vulgaris* rarely sets viable seed in Churchill, seeds for our experiment instead were collected from two nearby Ontario populations in late autumn 2020: the Koffler Scientific Reserve in Newmarket (44° 2′ 5″N, 79° 32′ 25″W), and Kipling subway station (43° 38′ 26″N, 79° 32′ 27″W). Seeds were used instead of rhizomes or roots to eliminate root size/structure variation and to reduce contamination that occurs with root biota. Existing northern populations are presumably derived from such temperate genotypes. Seeds were removed from capsules and mixed, and then rinsed in a distilled water bath, surface sterilized using 70% ethanol, and rinsed again to remove excess alcohol. Seeds were placed on moist filter paper in a petri dish wrapped in parafilm, and stratified at 5 °C for eight weeks prior to planting (Nadeau et al. 1992). During this time, seeds were rehydrated if necessary, and any mold was removed by alcohol. After stratification, seeds were planted on moist filter paper. Upon radicle growth, germinated seeds were planted in sterile potting soil. After further growth, plants at the cotyledon stage (2–4 leaves) were transferred into conical containers (SC10 Ray Leach Super Cell, Stuewe & Sons). Conical containers were separately suspended on a holding tray, and every other holding cell was left empty so that there was an equal amount of space between all containers. Any individuals that did not survive three days post-transfer were replaced once.

From each field soil sample, 50 mL was sieved and double-sterilized using an autoclave under a gravity cycle (referred to as "sterile"); a second 50 mL of soil sample was sieved but not sterilized (referred to as "live"). Ten mL of sterilized or unsterilized soil was then used to inoculate each of four replicates of approximately 230 mL of sterilized potting soil (2:1:1 ratio of soil, sand, and peat respectively, by volume). This proportion of inoculum represents about 4% of the volume of the container and is unlikely to introduce unwanted changes in the abiotic properties of the soil inocula (Vandegehuchte et al. 2010). Soil from different sites was not mixed in order to control for between-site variation (Reinhart & Rinella 2016), and inocula were randomized prior to being added into the sterile soil. This resulted in a total of 224 experimental units: 14 sites × 2 soil invasion statuses (invaded and uninvaded) × 2 sterility statuses (live and sterile) × 4 replicates.

For the first greenhouse iteration, individuals were left to grow for 6 weeks, and plant height (cm) was measured every seven days. This length of time was sufficient to allow plants to reach maturity and flower, which is desirable since effects of soil pathogens may change over maturation from seedling to adulthood (Moyano et al. 2021), soil pathogens may have variable effects on seed production (Dudenhöffer et al.

2018), and plants will eventually become rootbound. Plants were evenly watered every 3 days, and containers were shuffled every week within the greenhouse chamber. After 42 days, above-ground and below-ground plant material was harvested and dried at 60 °C for 48 h, and biomass was weighed (mg). Above- and below-ground biomasses were measured twice for each individual seedling; any repeated measurements that varied by over 2 mg was re-weighed. Plant height and biomass measurements were made while blind to soil treatment.

Serial inoculations were performed to document potential feedback (MacKay & Kotanen 2008; Brinkman et al. 2010; Gundale et al. 2019). After above- and below-ground plant material was harvested, 10 mL of sub-surface soil was collected from each conical container. Each new soil sample was used to inoculate 230 mL of sterile soil in a new conical container. Seeds originated from the same sources as iteration 1, and, once germinated, were planted into the containers, following the same methods as above. The same measurements were taken for this second iteration: height (cm) was measured every seven days, plant material was harvested and dried after 42 days, and above- and below-ground biomass (mg) was measured. This inoculation process and all measurements were then repeated for a third time. Note that soil samples were sterilized only prior to the experiment; "sterile" soils in iterations 2 and 3 were drawn from the previous "sterile" iteration, and thus, like the second and third iterations of the "live" treatments, may contain similarly greenhouse-acquired microbes.

Statistical analyses

All statistical analyses were completed using R (Version 1.4.1717). Since soil nutrient data were not normally distributed, the non-parametric Wilcoxon signed rank test on paired samples from the package 'rstatix' was used to compare soil properties (carbon, nitrogen, phosphorous, pH). Soil properties were then analyzed with correlation-based principal component analysis (PCA) from the package 'stats,' and visualized with the two first principal components. To determine whether mortality was equal between treatments and iterations, a Pearson's chi-square goodness of fit test was performed using 'rstatix.' Plant-soil feedback data were analyzed using linear mixed effects models from the package 'lme4' to assess the effect of live or sterilized invaded versus uninvaded soils on *L. vulgaris* growth. Model selection, using Akaike's Information Criterion corrected for small sample sizes (AIC_c), was conducted using the 'MuMIn' package. The linear mixed effects model providing the best fit to the data had the lowest AIC_C for all three iterations: biomass = invasion status × sterile status + initial height + (1|site). Invasion history and sterile status were treated as fixed effects, with initial height as a covariant to account for differences between individual plants, and site was treated as a random effect with separate intercepts to account for variation between sites. Type III Sums of Squares were used to test the overall effect of each variable. Note that for each of the four sterilization and invasion combinations in one iteration, there is a maximum of 56 individuals (14 sites × 4 replicates); a lower *N* indicates higher mortality.

Results

Soil nutrients

Analyses of the soil nutrient and pH data showed no significant ($p < 0.05$) differences between invaded and uninvaded plots, but Wilcoxon signed rank tests found evidence of a strong tendency ($0.1 > p > 0.05$) for invaded soils to contain more carbon ($W_{13} = 74$, $p = 0.050$) and nitrogen ($W_{13} = 62$, $p = 0.078$) (Fig. 2, Table 1). There were no differences in phosphorous (mg/kg dry) ($W_{13} = 58$, $p = 0.402$), or pH ($W_{13} = 34$, $p = 0.268$) between invaded and uninvaded plots. Note that these soil chemical analyses only show total carbon, nitrogen, and phosphorous, and may not reflect available forms.

Two major axes in the PCA explained 84.93% of the soil nutrient and pH variation between invaded and uninvaded sites (Fig. 3). Soil carbon, nitrogen, and phosphorous had negative loadings on component 1, while average pH had positive loadings; average pH and soil phosphorous had positive loadings on component 2 (Table 2). Invaded plots tended to score lower values on Axis 1, suggesting slightly higher nutrient levels on average, but extensively overlapped with uninvaded plots. Invaded and uninvaded soils did not cluster separately.

Plant mortality and growth

The effects of soil sterilization and invasion status did not affect total biomass in iteration 1 or in iteration 2 (Table 3). Interaction plots, which graph the interactive effect of invasion history and sterilization, also suggest no effect of soil sterilization treatment or invasion in iteration 1 or in iteration 2, though there was a non-significant ($p > 0.22$) tendency towards higher biomass in sterilized uninvaded soils in iteration 1 (Fig. 4). Iteration 1 tended to have highest average biomass (live + invaded ($N = 46$): 130.85 mg; live + uninvaded ($N = 46$): 157.21 mg; sterile + invaded ($N = 44$): 142.14 mg; sterile + uninvaded ($N = 48$): 163.98 mg) but differences were not significant; iteration 2 had the lowest biomass and greatest mortality (live + invaded ($N = 39$): 56.59 mg; live + uninvaded ($N = 40$): 58.90 mg; sterile + invaded ($N = 41$): 65.84 mg; sterile + uninvaded ($N = 43$): 76.39 mg). The difference in

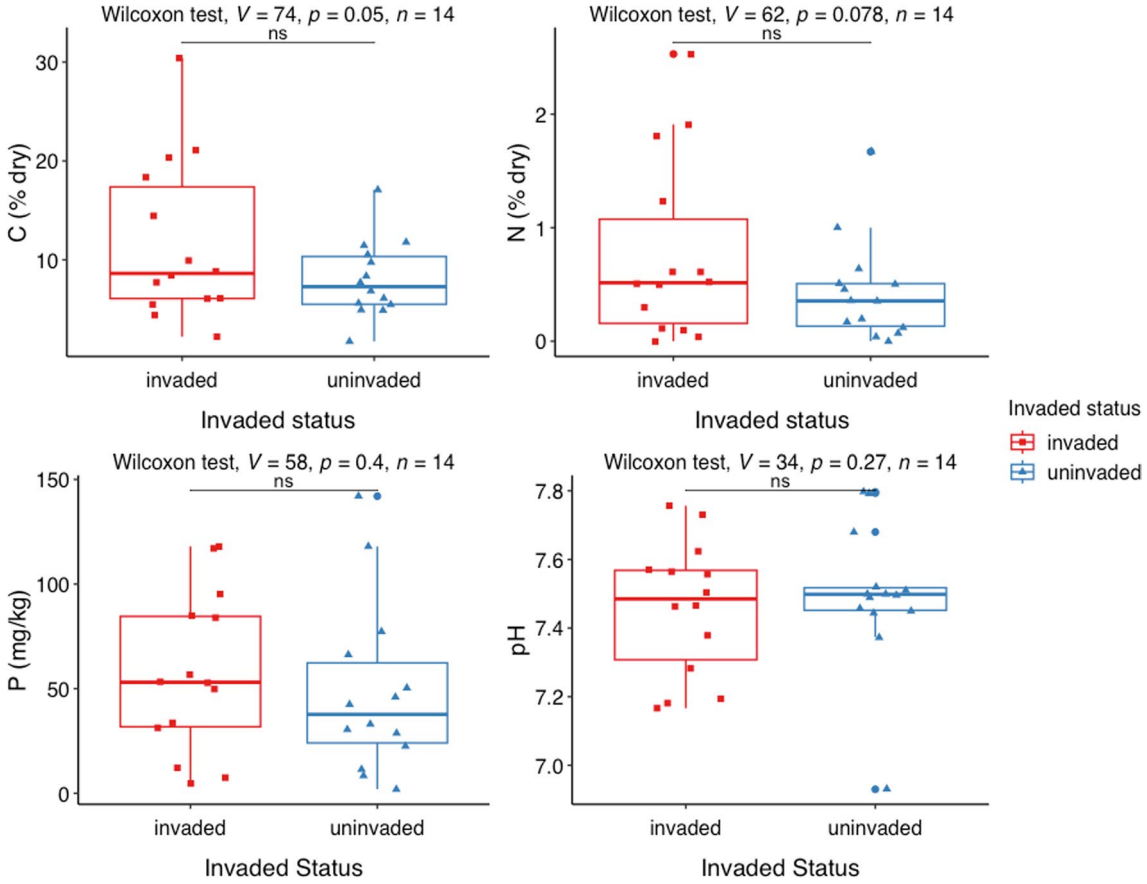

Fig. 2 Median, 25th and 75th percentile of carbon (C; % dry), nitrogen (N, % dry), phosphorus (P, mg/kg dry), and average pH in soil samples collected from plots invaded by *L. vulgaris* (red, squares) and uninvaded plots (blue, triangles). Statistics from paired non-parametric two-sided Wilcoxon tests comparing invaded and uninvaded plots reported; boxplots connected by a line are not significantly different

Table 1 Results from paired Wilcoxon signed rank tests comparing carbon, nitrogen, and phosphorous in the soil between invaded and uninvaded plots

	Invaded ($n=14$)		Uninvaded ($n=14$)				
	Mean	SD	Mean	SD	*df*	*W*	*p*
Carbon (% dry)	11.700	8.071	8.017	3.861	13	74	0.050
Nitrogen (% dry)	0.770	0.793	0.435	0.448	13	62	0.078
Phosphorous (mg/kg dry)	57.184	38.003	48.478	40.713	13	58	0.402
pH	7.460	0.195	7.496	0.2070	13	34	0.268

mortality was not significant between treatments (X^2 (11, $N=672$) = 16.984, $p=0.1084$). However, mortality was significantly different between the iterations on average (X^2 (2, $N=672$) = 11.875, $p=0.0026$). In iteration 3, biomass depended on both treatment and invasion status (Table 3). Overall, biomass on average was significantly higher ($p=0.01$) in previously sterilized soils (108.0 mg) than in inoculated live soils (93.3 mg) (Fig. 4). Biomass also tended to be slightly higher in uninvaded soils than invaded soils ($p=0.09$). Importantly, the interaction between sterility and invasion was significant ($p=0.02$): sterilization significantly increased *L. vulgaris* biomass in invaded soils (invaded + live ($N=52$): 81.42 mg; invaded + sterile ($N=49$): 120.63 mg) in comparison with biomass in uninvaded soils (uninvaded + live ($N=45$): 108.07 mg; uninvaded + sterile ($N=45$): 92.85 mg).

Discussion

The ability of a non-native species to modify the soil biota in its invaded range may influence its invasiveness. Our study found marginally significant differences in soil chemistry between soils invaded by *L. vulgaris* and uninvaded soils. In addition, we found evidence that negative

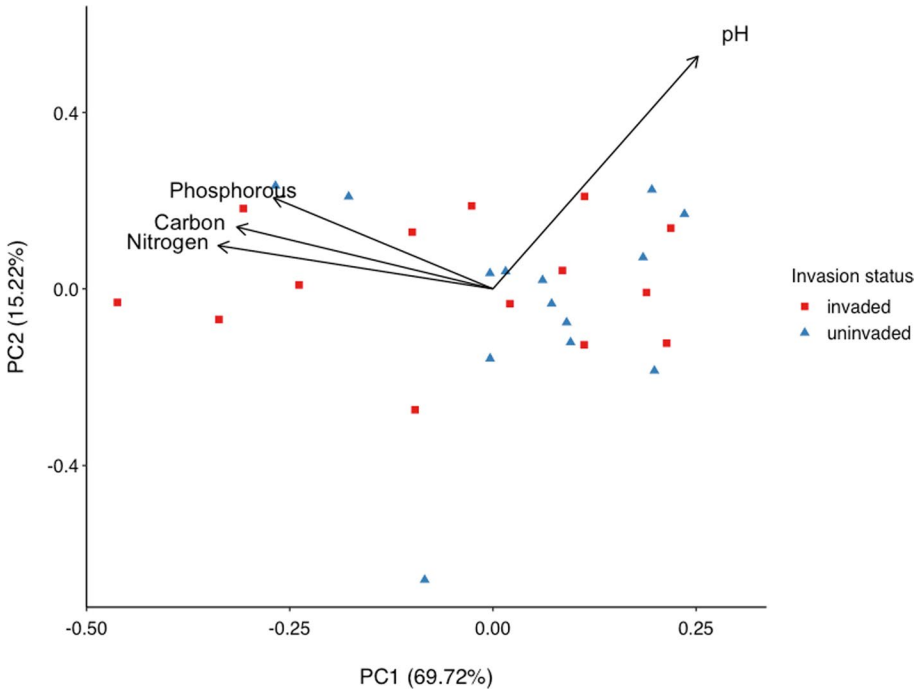

Fig. 3 Loading plot of the first two components of a PCA from soil chemical analysis data of 14 invaded (red, squares) and 14 uninvaded plots (blue, triangles)

Table 2 Soil chemical analyses loadings on four principal components axes. Largest |loading| for each PC indicated in bold

	PC1	PC2	PC3	PC4
Nitrogen (% dry)	**−0.5713**	0.1657	−0.2459	**−0.7653**
Average pH	0.4264	**0.8914**	−0.1284	−0.0840
Phosphorus (mg/kg)	−0.4561	0.3488	**0.8033**	0.1579
Carbon (% dry)	−0.5326	0.2372	−0.5270	0.6184

plant-soil feedbacks became stronger in invaded soils than in uninvaded soils. Since there was no evidence of greater resistance by uninvaded soils, these results suggest that feedbacks do not explain the persistent failure of *L. vulgaris* to spread and consequently invade natural communities in Churchill; instead, the explanation must lie somewhere else.

In iterations 1 and 2, we found no evidence that soil biota initially affected the performance of *L. vulgaris* in either invaded or uninvaded soils. We did, however, find evidence that negative feedbacks developed over time. By iteration 3, sterilization improved plant performance more for invaded soils than for uninvaded soils, indicating stronger negative feedback in invaded soils. We conclude that populations of *L. vulgaris* persist despite negative feedback, not because of an absence of it. These results agree with numerous studies that suggest invaders can experience negative feedbacks in invaded soils (Reinhart & Callaway 2006; Gundale et al. 2014). Although we cannot guarantee the absence of cross-contamination, such

Table 3 Results from a linear mixed effects model testing the difference of total *L. vulgaris* biomass in all three iterations between soil treatments, with "Invaded status" and "Sterile status" as fixed effects, initial height as a covariant, and site as a random effect

Predictors	Estimates	X^2	*df*	*p*
Biomass (iteration 1)				
Intercept	4.8311	0.1115	1	0.7385
Invaded status	−0.5505	0.0012	1	0.972
Sterile status	−3.7230	0.0556	1	0.814
Invaded status * Sterile status	26.9240	1.4749	1	0.226
Initial height	97.5542	201.8567	1	**$< 2^{-16}$**
Biomass (iteration 2)				
Intercept	−36.5203	6.8059	1	**0.0091**
Invaded status	1.584	0.0205	1	0.88645
Sterile status	5.127	0.2164	1	0.64245
Invaded status * Sterile status	3.381	0.0479	1	0.82697
Initial height	119.751	64.7780	1	**$< 1.89^{-13}$**
Biomass (iteration 3)				
Intercept	−29.4839	2.1387	1	0.1436
Invaded status	25.1251	2.9782	1	0.0862
Sterile status	39.0066	7.5158	1	**0.0061**
Invaded status * Sterile status	−48.6573	5.8094	1	**0.0191**
Initial height	133.1123	41.2464	1	**$< 1.342^{-10}$**

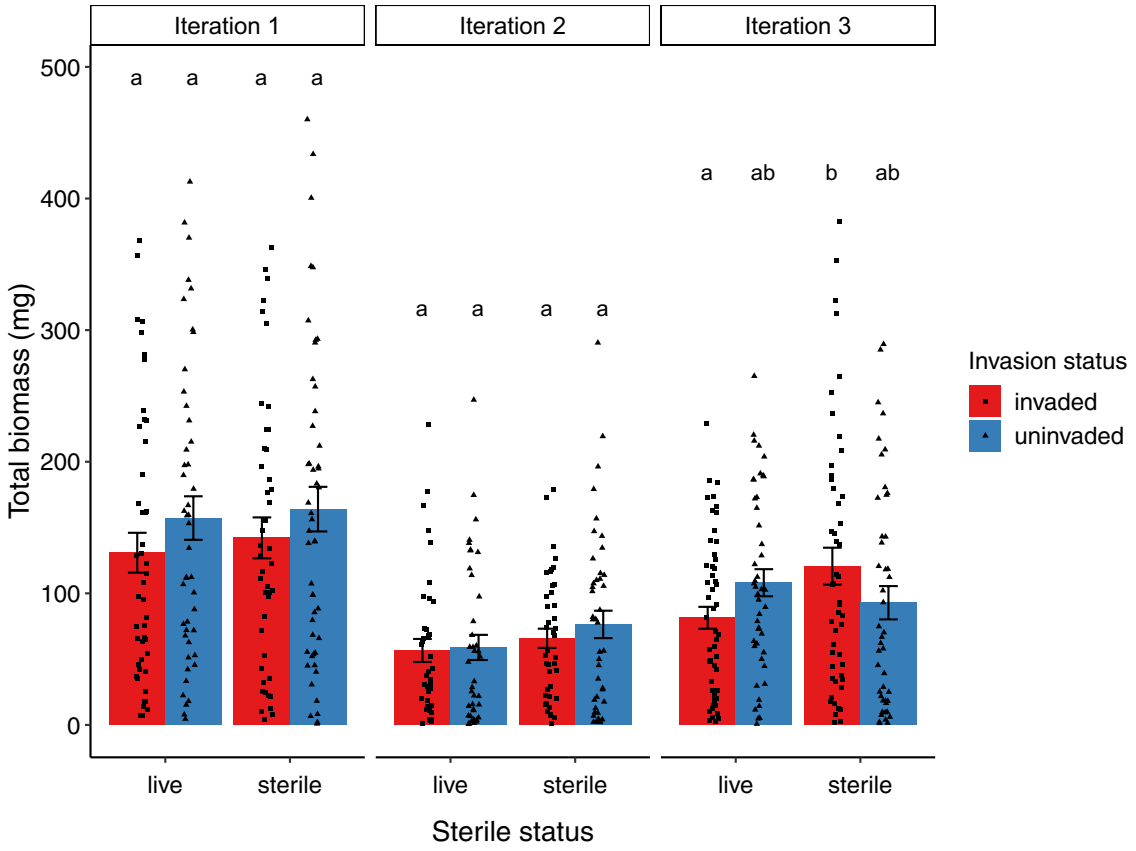

Fig. 4 Average biomass (mg) of *L. vulgaris* grouped by greenhouse iteration, separately for invaded (red bars) and uninvaded soils (blue bars). Individual plant biomass in invaded soils (squares) and uninvaded soils (triangles), with standard errors are indicated. Within each iteration, bars sharing the same letter do not differ significantly ($p > 0.05$; multiple comparisons on a linear mixed effects model). In iterations 1 (left) and 2 (middle), there were no significant effects of invasion, sterility, or their interactions ($p > 0.2$; Table 3) on total biomass. In iteration 3 (right), live soil inhibits growth more when sampled from invaded plots than when sampled from uninvaded plots (invasion × sterility interaction $p < 0.02$; Table 3), indicating negative feedback is stronger in invaded soils

contamination could not explain the observed differences between treatments. Differences in mortality between iterations may be due to seasonal differences in daylight and growing conditions in the greenhouse; this is one reason we chose to analyze each iteration independently as an internally consistent trial.

There was a tendency for invaded soils to contain more carbon and nitrogen. These results are similar to those of Kent et al. (2018), who found higher levels of ammonium and phosphorous (but not nitrate) in sites that contained invasive species versus sites that were uninvaded. However, any differences in these elements were weak, and multivariate analyses indicated substantial overlap between invaded and uninvaded sites. These results do not suggest that substantial chemical differences between soils explain their invasion status, as might be expected given that paired plots were chosen to be as similar as possible; however, it is possible that *L. vulgaris* itself may modify the chemistry of plots where it is established, as has been shown for other invaders (Liao et al. 2008; Ehrenfeld 2010; Gibbons et al. 2017; Zhang et al. 2019).

Measuring effects of the entire soil community integrates the effects of both antagonists (nematodes, bacteria, and fungal pathogens including inappropriate mycorrhizal partners) and mutualists (mycorrhizae, beneficial rhizosphere bacteria) in soil, and invasive species can depend on mutualistic microorganisms, such as mycorrhizal fungi, in the invaded range (Moyano et al. 2021). Therefore, the relatively more positive effect of sterilization in invaded soils compared to uninvaded soils in principle could result from either the removal of soil pathogens from invaded soils or the removal of mutualists from uninvaded soils. Additionally, although sterilization via autoclaving may release additional nutrients into soils (Endlweber & Scheu 2006; Dietrich et al. 2020), we added only a small percentage of soil inoculate (4% of the volume of the container) to the sterilized potting soil for each replicate to minimize any potential effects of changes in soil properties (Vandegehuchte et al. 2010). In fact, trendlines could suggest a slight decline in growth with

sterilization in uninvaded plots vs an increase in invaded plots (Fig. 4). Since *L. vulgaris* relies on relatively generalist AM mycorrhizae (Pendleton and Smith 1983), it is likely that some suitable mycorrhizal partners occur in uninvaded habitats, but it seems unlikely that such mutualists would be more common in these largely ericoid-mycorrhizal landscapes than in invaded soils. Consequently, we believe our results are more likely to be driven by species- or habitat-specific pathogens than specialized mutualists.

At the range edge, several factors may be influencing the invasion success of non-native species. Our results suggest that negative plant-soil feedbacks may not be limiting the spread of *L. vulgaris* in Churchill. Instead, a factor currently reducing local spread of *L. vulgaris* likely is propagule limitation: lack of seed production in our study area currently may slow colonization of suitable sites. Ongoing climatic warming may change this situation by altering flowering phenology (Mulder et al. 2017), allowing seed maturation in at least warmer years; evidence from recent field seasons suggest that some *L. vulgaris* seeds are able to fully mature in late autumn (*pers. obs.*), though their germination success remains low. Our results suggest that seeds of *L. vulgaris* should be able to germinate and grow in soils where it currently is absent; in fact, colonization of new areas may help *L. vulgaris* escape negative feedbacks in already-occupied sites. In future, increased seed production and successful dispersal may transform this plant into a more aggressive invader, as is the case in southern Canada (Saner et al. 1995).

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analyses were performed by V.M. Zhang. The first draft of the manuscript was written by V.M. Zhang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets and code generated during the current study will be made publicly available on Dryad after the manuscript is accepted. The datasets and code are currently available from the corresponding author upon reasonable request.

Declarations

Competing interest The authors have no competing financial or non-financial interests to declare.

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