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Research Article

Vascular plant diversity in Swedish road verges of high conservation value is threatened by the invasive alien herb *Lupinus polyphyllus* Lindley

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Road verges can be important habitats for vascular plant communities and the organisms that, in turn, depend on them. However, the plant diversity in Swedish road verges is threatened by the invasive perennial plant *Lupinus polyphyllus* Lindl. Therefore, this study aimed to investigate the effects of *L. polyphyllus* on species richness, diversity, and biotic and abiotic characteristics of dissimilar plant communities in road verges. The study was performed in 24 road verges of high nature value (i.e. with high flowering plant diversity) in central Sweden. We selected road verges within two study areas, one dominated by forest (west) and the other by agricultural land (east). In each road verge we established a pair of 1 m^2 plots. One plot was dominated by *L. polyphyllus* while in the other plot *L. polyphyllus* either had very low occurrences or was completely absent. We investigated whether species richness, effective number of species, and the probability of occurrence of species belonging to three functional groups (based on their competitive ability and affiliation to nutrient rich soil) differed between plots with vs without lupine and if responses varied between the study areas. We found that *L. polyphyllus* reduced species richness and diversity of plant communities in road verges. However, we found that even though the direction of the effect was similar, the magnitude was strongly dependent on the pre-existing communities. Community composition differed between the study areas. The study area in the west hosted a lower proportion of competitive species typical for nutrient poor soils compared to the east. We conclude that invasion by *L. polyphyllus* is a serious threat to vascular plant communities but that the response is context dependent. Communities with high abundance of vulnerable and poor competitive plant species should be prioritised for eradication and control of the invasive.

Keywords: Biodiversity conservation, community ecology, functional groups, invasive alien plants, road verge habitats, semi-natural grasslands

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Introduction

Invasive alien species (IAS) are considered one of the major threats to global biodiversity together with habitat loss, over-exploitation, pollution, and climate change (Purvis et al. 2019, Roy et al. 2023). Furthermore, IAS may have negative impacts on food security, affect human health, reduce ecosystem services, and their management and control may cause large monetary costs to society (Rai and Singh 2020, Diagne et al. 2021). Despite there currently being little evidence showing that plant invasions can directly lead to extinctions of resident species (Downey and Richardson 2016), invasive alien plants (IAP) can have significant impacts at the species, community, and ecosystem level (Pyšek et al. 2020). IAP can reduce the abundance and diversity of resident species, modify soil seed banks, can often increase productivity, nitrogen (N) availability, as well as phosphorus (P) pools, and can cause regime shifts that may be impossible to reverse (Vilà et al. 2011, Pyšek et al. 2020).

Road verges have been demonstrated to serve as conduits for the expansion of IAP (Christen and Matlack 2009, Lemke et al. 2021). Yet, the vegetation in these novel habitats can closely resemble that found in semi-natural grasslands traditionally managed through mowing or grazing, and without input of fertilisers or pesticides i.e. semi-natural grasslands (Vanneste et al. 2020, Dániel-Ferreira et al. 2023). Road verges with a high diversity of plant species (i.e. of high nature value) can be particularly important because they provide temporal stability in the availability of floral resources — an important factor for pollinators and the service they provide (Dorado and Vázquez 2014). Furthermore, road verges can constitute complementary habitats, refuges, and dispersal corridors for threatened grassland species (Auestad et al. 2011, Helldin et al. 2015, Oldén et al. 2021). However, the potential of road verges to contribute to biodiversity conservation may be deteriorated by the presence of IAP. In a recent report by the Swedish Transport Administration (Sjölund 2021) it was found that 60% of a total of 4750 road verges of high nature value (~ 20 000 km and 6000 ha) were threatened by IAP, in particular by *Lupinus polyphyllus*.

Lupinus polyphyllus (garden lupine) is considered one of the most challenging non-native plant species in Europe (Tyler et al. 2015, Nentwig et al. 2018). The species has been documented in a wide range of habitats, including nutrient-rich and nutrient-poor road verges (Eckstein et al. 2023). In *Nardus*-grasslands dominated by *L. polyphyllus*, the vertical structure of the vegetation undergoes a reversal, leading to a reduction in the number and abundance of low-statured species in favour of the large, competitive grasses and ruderal herbs (Otte and Maul 2005, Thiele et al. 2010). Consequently, *L. polyphyllus* may also alter community composition, resulting in species turnover, and it may also contribute to the observed homogenization of community composition (Valtonen et al. 2006, Hansen et al. 2021). This, in turn, leads to increased community-weighted means of canopy height, specific leaf area, and decreased leaf dry matter content (Hansen et al. 2021). Furthermore, *L. polyphyllus* has the ability to fix atmospheric nitrogen with the help of symbiotic bacteria (diazotrophs) in root nodules and can thus significantly increase soil N pools (Vilà et al. 2011), which can also lead to alterations to the native community composition. In general, *L. polyphyllus* is widely recognised for its negative impacts on plant species diversity within invaded communities (Valtonen et al. 2006, Thiele et al. 2010, Ramula and Pihlaja 2012, Hansen et al. 2021).

The extent of *L. polyphyllus*' impact on species diversity varies among studies and appear to be context dependent (Eckstein et al. 2023). This contextual dependency arises from different invasion drivers operating at different spatial scales (Catford et al. 2019, Czarniecka-Wiera et al. 2020). At the local scale, biotic interactions and microclimatic conditions are more relevant, while at larger spatial scales the structure of the landscape, temperature, and precipitation become more important (Czarniecka-Wiera et al. 2020). The high level of uncertainty and limited predictability of invasions resulting from a strong context-dependency has hindered the adoption of measures aimed at controlling and mitigating the spread of IAP (Latombe et al. 2019).

Rather few studies of effects of IAP on biodiversity are performed in road verge communities (but see Valtonen et al. 2006, Ramula and Pihlaja 2012). Given the widespread presence of *L. polyphyllus* in Swedish road verges, and the acknowledged context-dependency of IAP effects, it is important to develop the capability to forecast the impact of invasive species on biodiversity in road verges of high conservation value. Furthermore, it is essential to estimate the extent to which these effects may vary across dissimilar road verge communities. Such knowledge can help to identify the circumstances in which eradicating and preventing the spread of IAP will have the most beneficial outcome for preserving native species.

We aimed to explore effects of *L. polyphyllus* on dissimilar plant communities in road verges of high nature value by comparing species richness and diversity between plots dominated by the invasive and plots without it. We predicted that 1) *L. polyphyllus* has negative effects on vascular plant species richness and diversity, 2) the direction and magnitude of the effect will depend on the proportion of plant species that are poor or strong competitors in the community, and 3) *L. polyphyllus* has effects on biotic and abiotic community characteristics, such as litter depth, community weighted vegetation height, and indicator values for light, nutrients, soil moisture, and soil reaction.

Methods

Study design and data collection

We performed the study in 24 road verges of high nature value in two study areas in south-central Sweden (Fig. 1a, Supporting information). Selected road verges in the western study area were mostly in forested landscapes dominated by till soils. The road verges in the eastern area were

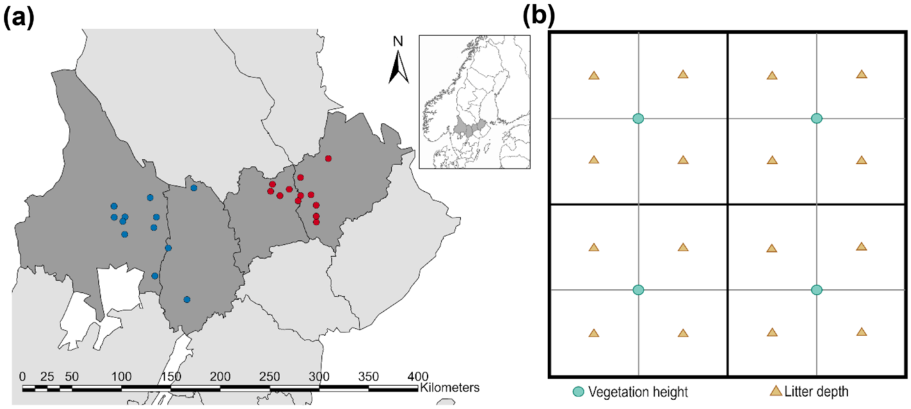

Figure 1. a) Location of the 24 road verges used in this study. Road verges in the west area are marked with blue and in the east are marked with red. b) Illustration of the measurements taken in each plot. Two of these plots were placed in one site. One of the plots was dominated by lupine, while the other was not.

predominantly in agricultural landscapes with more clay soils. We expected the plant communities to differ between study areas, which later proved to be the case. For simplicity, the road verges in the forested landscapes are hereinafter referred to as the western area, and the road verges in the agricultural landscapes are referred to as the eastern area. Road verges of high nature value were selected using the Swedish National Road Database (NVDB: www.nvdb.se). Road verges that are classified as 'of high nature value' by the Swedish Transport Administration in this database fulfil at least one of four of the following conditions in terms of plant species, they: 1) hold indicator, rare, or threatened species, 2) have special species composition and/or particularly high species richness or frequency of indicator species, 3) provide an important ecological resource (e.g. host plants), or 4) promote species' dispersal and landscape connectivity (Lindqvist 2012).

In each of the road verges, we selected one stretch which fulfilled two criteria: 1) it had homogenous environmental conditions (soil type, sun exposure, adjacent habitat) and community composition (*L. polyphyllus* not included), based on a visual inspection; 2) it had *L. polyphyllus* stands in parts of the stretch, of which at least one stand was dense. In the selected road verge stretch we placed one pair of 1-m^2 plots. One plot was placed in the centre of a vegetation stand dominated by *L. polyphyllus* (hereafter referred to as 'lupine dominated'). A plot dominated by lupine was characterized by the presence of the invasive in at least twelve out of a total of sixteen subplots (at least 75% of the 1-m^2 plot covered by *L. polyphyllus*). The other plot was placed in the same road verge stretch and as close as possible to the first plot, but in a part of the stretch where *L. polyphyllus* did not occur or had very sparse occurrence of small, newly established plants (hereafter referred to as 'without lupine'). Other IAP did not occur in the plots, except for a few shoots of *Solidago canadensis* in some plots.

As the data were collected separately in each study area, there were some slight differences in the placement of the plots. In the western area, the absence of *L. polyphyllus* in the plots without lupine was prioritised. The plot was therefore placed as close as possible to the lupine dominated plot in the same road verge, but not exactly adjacent to it as it was done in the eastern area. In the eastern area, a few small shoots of lupine were accepted in the plot 'without lupine' in order to always place the two plots in a pair directly adjacent to each other. Since those shoots were young, small and recently established, they were not considered to influence the plant community. In both study areas, the plot layout aimed at ensuring that the two plots in a pair had similar community composition prior to the establishment of *L. polyphyllus*.

After selecting the position of the plots, each plot was divided into 16 sub-plots of 625 cm^2. All vascular plant species, except for species belonging to the family Poaceae (true grasses), per plot were identified to species level (for some apomictic *Hieracium* to species group) and each species' abundance was estimated as the number of subplots in which the species was present. A species could thus have a maximum of 16 occurrences per plot. Members of the family Poaceae were not recorded because it would have been too time consuming and inaccurate to try to identify all leaves and vegetative plants of grasses. The nomenclature used follows the Swedish Species Information Centre taxonomic database Dyntaxa (http://www.slu.se/dyntaxa/).

A compound estimate of vegetation height and density was obtained using a grassland ruler (Ekstam and Forshed 1996) in the centre of the four 0.5 × 0.5 m squares within each plot (Fig. 1b). We also measured litter depth in the centre of each sub-plot and used the mean of the 16 measurements as average litter depth per plot. Litter depth was measured as the distance between the surface of the litter layer and the surface of the soil, with an accuracy of 5 mm. The road verges were surveyed between 2 June and 17 July, 2020. None of the road verges had been mowed at the date of the survey.

Analyses

All analyses were performed in R ver. 4.1.2 (www.r-project.org). We used generalized linear mixed effects models (GLMMs) using the package 'glmmTMB' (Brooks et al.

2017) to examine changes in species richness (the number of vascular plant taxa per quadrat, excluding true grasses and woody plant species) and the effective number of species (the number of equally common species that would be needed to generate the given Shannon index, Chao et al. 2014) related to the presence of the invasive species *L. polyphyllus* in road verges. The effective number of species is given by:

$$^{1}D = e^{H'} = e^{\left(-\sum_{i=1}^{S} p_i \log p_i\right)}$$

Where p_i is the relative frequency of each species ($p_i = N_i/N$), *S* corresponds to the observed number of species, and ^{1}D is the equivalent to the exponential of the Shannon entropy measure of diversity (H′), which weights each species by its relative frequency (i.e. Hill numbers with $q = 1$). The effective number of species was calculated using the package 'vegan' and the function *renyi* with the argument 'hill' set as true (Oksanen et al. 2020). The models used species richness and the effective number of species as response variables to the presence of *L. polyphyllus* in the plot (factor: present (yes) or absent (no)). To detect differences in the effect of *L. polyphyllus* between the plant communities in the studied areas, study area (factor: eastern or western area) was added as an interacting factor with the presence/absence of *L. polyphyllus* in the plot. Finally, a random effect for site was added to control for the repeated measures per site. Species richness was modelled with a Poisson distribution with a log link, whereas the effective number of species was modelled with a Gaussian distribution and a log link. To test the importance of the interaction and of the explanatory variables, we calculated the AICc values for two additional candidate models: one without the interaction between the type of plot and the study area, and another for the null model. This was done for each model separately, once for the model for species richness model and another for the effective number of species. The residual diagnostics for the two models described above were visually inspected using the package 'DHARMa' (Hartig 2020). Finally, we computed spline correlograms (function *spline.correlog* in the 'ncf' package, Bjørnstad and Falck, 2001) of the models' residuals (bootstrap confidence intervals based on 1000 resamples) to check for the absence of spatial autocorrelation.

To explore whether the composition of vascular plant communities between plots dominated by *L. polyphyllus* and plots without the invasive species were (dis)similar, we performed a principal coordinate analysis (PcoA) ordination based on Bray–Curtis distance matrices. The matrices were calculated using the '*beta.pair.abund*' function from the package 'betapart' (Baselga and Orme 2012), and the *betadisper* function from the 'vegan' package (Oksanen et al. 2020) was used to test for homogeneity of variances within plot types using the entire dataset and for each region separately. We excluded *L. polyphyllus* from the ordination given the design of the study. Then, to determine whether there were any statistically significant differences in community composition between plots dominated by lupine and plots without, we performed a PERMANOVA (function *adonis2* in 'vegan') using the same explanatory variables as before; the presence of lupine in the plot (factor: yes or no) and the study area (factor: east or west). Finally, we computed a spline correlogram based on the Bray–Curtis distances among species' assemblages and the plot coordinates to check for spatial autocorrelation among the species compositions.

To further explore the effects of *L. polyphyllus* on native plant communities, the species were grouped into four functional groups describing the species' relationships to competition and soil nutrient levels: very poor competitors and nitrophobous (hereafter denoted very poor competitors), poor competitors and slightly nitrophobous (poor competitors), moderate competitors and moderately nitrophilous (moderate competitors), and strong competitors and nitrophilous species (strong competitors). This classification was performed in three steps. First, the species were sorted into groups based on their size and their habitats in central Sweden. Habitat information for each species was compiled using regional floras that cover the study area (Malmgren 1982, Larsson and Danielson 1994, Jonsell and Aronsson 2010, Löfgren 2013). A total of 21 groups were identified (Supporting information). Second, based on habitat and plant size (from the first step), each species was assigned a degree of competitiveness ranging from 0 (very bad competitor) to 3 (strong competitor), and affiliation to nutrients: 0 for very nitrophobous species and 3 for nitrophilous. Third, the sum of these estimates (thus ranging from zero to six) was used to assign the species to one of the four functional groups mentioned above. The functional group very poor competitors included small species from dry, often sandy habitats, especially those where some ground disturbance occurs. Examples are *Rumex acetosella*, *Pilosella officinarum* and *Scleranthus perennis*. The group poor competitors included small species from low-productive semi-natural grassland and other low-productive open or semi-open habitats. Examples are *Primula veris*, *Anthyllis vulneraria* and *Trifolium spadiceum*. The group moderate competitors included small or rather small species from somewhat more nutrient-rich habitats than the previous group: various semi-natural grasslands, field margins, and arable fields, as well as medium-sized species from rather dry and poor, often ruderal habitats. Examples are *Stellaria graminea*, *Geum urbanum* and *Campanula patula*. The group strong competitors, finally, included large or rather large species usually occurring in habitats that are either nutrient-rich or with tall vegetation, or both, such as strongly fertilised arable fields and abandoned grassland. Examples are *Anthtriscus sylvestris*, *Cirsium arvense* and *Galium mollugo*.

Using this classification, we investigated whether the invasive *L. polyphyllus* influenced the proportion of vascular plants belonging to each functional group in a plot by fitting a baseline-category logit model using the function *mblogit* from the package 'mclogit' (Elff 2022). The model followed a similar structure as the models described above, in which we included an interaction between the study area and the presence/absence of the invasive species in the plot. The total occurrences in each category for each site were added as weights, and a random effect for site was added to control for the repeated measures per site. For analysis purposes, the functional groups 'very poor competitors' and 'poor competitors' were pooled together into one group 'poor competitors'. Finally, we performed a post-hoc test using the 'emmeans' package (Lenth 2020) to explore whether the differences in the proportions of species in each functional group were different between plots and regions. The p-value was corrected for multiple comparisons using the Tukey method. The residual diagnostics for the two models described above were visually inspected using the package 'DHARMa' (Hartig 2020).

To explore the mechanisms by which the invasive *L. polyphyllus* potentially influence species richness and community composition of vascular plants, we compared vegetation height and litter depth between plots with versus without lupine using paired t-tests. We also performed paired t-tests between plots with versus without lupine for the community weighted (weighted by species frequencies) species-specific plant height and indicator values for light, moisture, soil reaction, and nutrients (lupine not included). The ecological indicator values of species were extracted from Tyler et al. (2021). We used community weighted means to control for the effect on the mean of the most dominant species, using the function *weighted.mean* which uses the abundance of each species as weights. Species-specific plant heights were taken from a Swedish flora (Mossberg and Stenberg 2010), excluding woody species since these trees and shrubs will not reach their natural size in road verge habitats.

Results

We recorded a total of 127 vascular plant taxa (species and species groups) in the road verges. *Achillea millefolium*, *Ranunculus acris subsp. acris*, and *Taraxacum ruderalia* group were the most common (Supporting information). The total number of plant taxa in the plots without *L. polyphyllus* in the eastern area was 79 while in the west it was 75. In plots dominated by *L. polyphyllus*, the total number of species in the east was 75, and only 40 plant taxa were found in these plots in the west. The average number of species in lupine dominated plots was 10.04 (min = 3, max = 24), while the average number of species in plots without lupine was 15.88 (min = 4, max = 25). Some species occurring in both areas were completely absent in the lupine dominated plots (e.g. *Veronica officinalis, Anemone nemorosa,* and *Prunella vulgaris*). Species accumulation curves indicated that species richness between plot types showed similar rates of species accumulation, meaning that the surveys between plots with and without lupin were comparable to each other (Supporting information). The difference in species richness between plots with and without lupine was marginally significant (Table 1), as well as the effective number of species (Supporting information, Table 2). However, the difference inspecies richness and the effective number of species between the plots with and without lupine was larger in the western study area than in the eastern area (Fig. 2, Tables 1 and 2, Supporting information). We found no evidence of spatial autocorrelation at any distance for the residuals of either of the models (Supporting information). The AICc values showed that the model with the interaction between the type of plot and the study area was the best for both analyses (Supporting information).

The PCoA analysis of community composition showed a general overlap of the plant communities in the plots with and without lupine (Supporting information). Furthermore, there were no differences in the variances (i.e. average distance to the corresponding centroid) for the plant communities in plots with and without lupine ($F_{1,46} = 1.52$, $p = 0.22$). As expected, the plant species compositions differed between study areas (Supporting information). The one-way ANOVA showed significant differences in the variances for each area ($F_{1,46} = 5.16$, $p = 0.03$), and the PERMANOVA analysis showed that while there were no differences in the community compositions between the plots with and without lupine, there were differences between study areas (Supporting information). Still, there were no differences in the variances between plots with and without lupine neither in the east ($F_{1,22} = 0.16$, $p = 0.69$; Supporting information) nor in the west ($F_{1,22} = 1.39$, $p = 0.25$; Supporting information). We found no evidence of spatial autocorrelation among community compositions at any distance (Supporting information).

The total number of occurrences of species in each functional group decreased in the plots dominated by lupine (Fig. 3). The multinomial logistic model and the post-hoc analysis showed that species that are poor competitors have a higher probability of occurring in plots without lupine than in plots dominated by the invasive, but this difference was significant only in the western study area (Fig. 4,

Table 1. Results of the generalized linear mixed model exploring the role of the invasive species *L. polyphyllus* (lupine) on the species richness of vascular plant communities in road verges. Shown are the chi-square values (χ^2), degrees of freedom (df), estimates (e), standard error (SE), and p-values (p). p-values in bold are significant at the 0.05 level or lower, and marginally significant effects are indicated in italics.

Predictor	χ^2 (df = 1)	e	SE	p
(Intercept)	.	2.76	0.10	.
Lupine (yes)	2.82	-0.18	0.11	*0.09*
Area (west)	0.15	-0.05	0.14	0.71
Lupine × Area	16.81	-0.71	0.17	**< 0.001**

Table 2. Results of the generalized linear mixed model exploring the role of the invasive species *L. polyphyllus* (lupine) on the effective number of species of vascular plant communities in road verges (Hill number, q = 1). Shown are the chi-square values (χ^2), degrees of freedom (df), estimates (e), standard error (SE), and p-values (p). p-values in bold are significant at the 0.05 level or lower, and marginally significant effects are indicated in italics.

Predictor	χ^2 (df = 1)	e	SE	p
(Intercept)	.	2.44	0.10	.
Lupine (yes)	3.64	-0.18	0.09	*0.06*
Area (west)	0.03	-0.02	0.13	0.86
Lupine × Area	12.43	-0.64	0.18	**< 0.001**

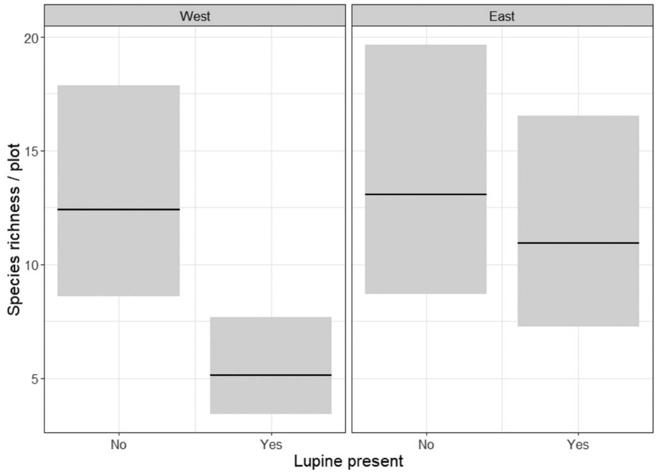

Figure 2. Average number of vascular plant species per plot in plots without (no) and with (yes) the dominant invasive *Lupinus polyphyllus*. The plots show average species richness per plot (black line) and the 95% confidence intervals (grey bands).

Supporting information). Strong competitive species were more commonly found in plots dominated by lupine than without lupine in both areas (Fig. 4). Species with intermediate competition capacity showed no significant response to lupine in either direction. The best candidate model included the interaction between the type of plot and the study area (Supporting information).

The mean vegetation height of vascular plant communities in lupine dominated plots was in average 42.5 cm higher than in plots without the invasive (Table 3, Supporting information). In contrast, the community weighted species-specific plant height was similar in plots with vs without lupine (Table 3, Supporting information). There were no differences between plots with vs without lupine in terms of litter depth (Table 3, Supporting information), but the average litter depth in the western region was in average ~ 0.82 cm thicker than in the eastern region (Table 3, Supporting information). There was no general correlation between vegetation height and litter depth (Pearson's $r_{46} = 0.16$, $p = 0.29$). However, in plots without lupine, vegetation height tended to be positively correlated to litter depth (Pearson's $r_{22} = 0.4$, $p = 0.05$) but this was not the case in the plots with lupine (Pearson's $r22 = 0.16$, $p = 0.44$). Regarding the ecological indicator values, we found that only nutrients (specifically nitrogen) differed between plot types ($t\ (23) = -2.22$, $p = 0.04$). We did not observe any significant differences between plots with and without lupine regarding indicator values for light, moisture, and soil reaction (Fig. 5). However, there were differences in between plot types for the indicator value for light in the east area but not in the west (Supporting information).

Discussion

Our results indicate that the invasive *L. polyphyllus* reduces species richness and diversity of vascular plants in road verges, and that the magnitude of the effect depends on the pre-existing community. By placing plots with and without

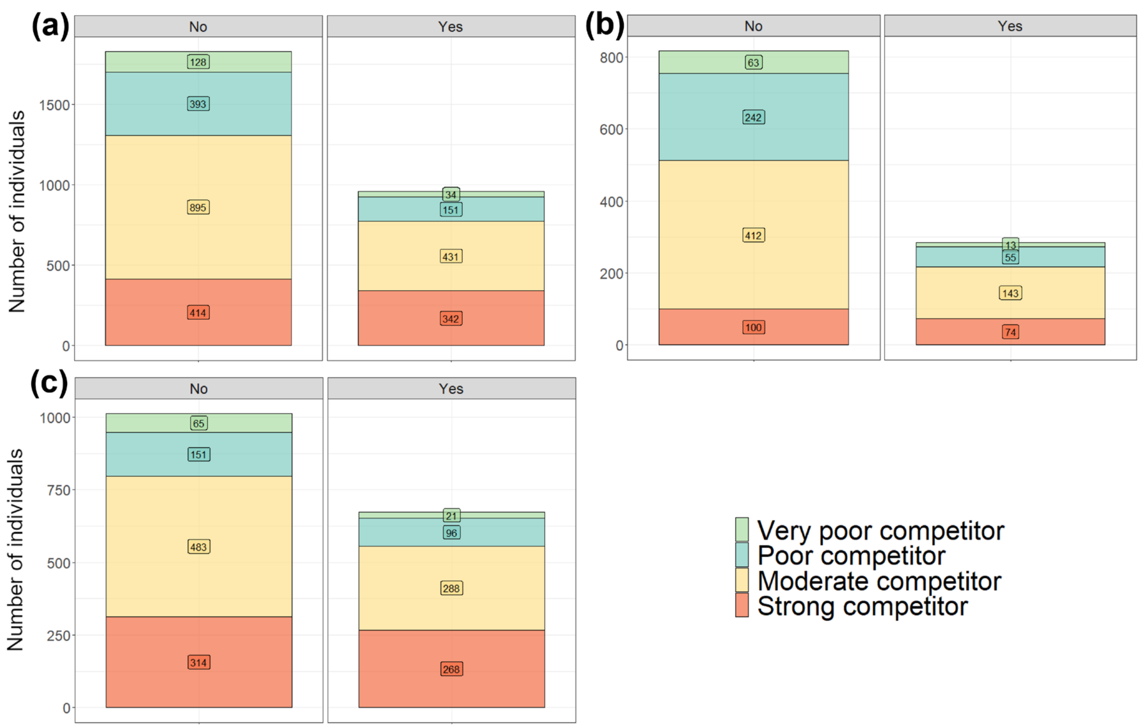

Figure 3. Total plant occurrences belonging to the four categories explained in the Supporting information in each type of plot for (a) the entire dataset, (b) the west area, and (c) the east area. No = plots without lupine, yes = plots dominated by lupine.

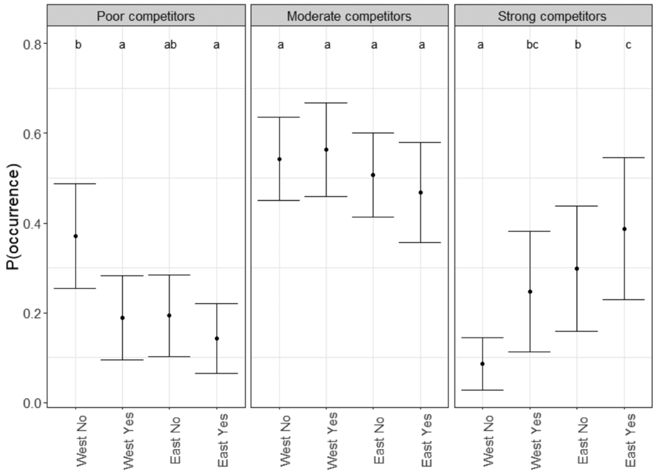

Figure 4. Probability of occurrence of species belonging to each of the functional groups. The lines represent the standard error. The letters on the top part of the panels are the results from the post-hoc analyses. Pairs that do not share a letter are significantly different from each other. No = plots without lupine, yes = plots dominated by lupine.

lupine close to each other in the same homogenous stretch of road verge and thus ensuring a similar community composition between plots with vs without lupine before invasion, we were able to explore the effects of *L. polyphyllus*. We found that plant species that exhibit poor competitive abilities were the most adversely affected. Consequently, communities characterized by a high proportion of poorly competitive plant species are particularly vulnerable to lupine invasion and should be given priority for eradication and control measures. In contrast, strong competitors were favoured by lupine, likely due to the nutrient enrichment that *L. polyphyllus* may contribute to. A group of species with intermediate competition capacity showed to be rather unaffected by lupine.

As predicted, the species richness of vascular plants was lower in lupine-dominated plots. This is in accordance with previous studies that demonstrated negative effects of the invader on species richness (Valtonen et al. 2006, Hejda et al. 2009, Hansen et al. 2021, Prass et al. 2022). Also variable effects of the invasive on different functional groups are in line with previous studies showing that invasion-diversity relationships may vary among different groups of resident species (Thiele et al. 2010) or among communities (Hansen et al., 2021). Given that lupine reduced the abundance of certain functional groups of plants, overall plant diversity was also reduced, which was more evident in the western than in the eastern area. The PCoA did not show any significant effects of *L. polyphyllus* on community composition along road verges. This is in line with Ramula and Pihlaja (2012) who studied meadows, forests, road verges and wastelands, whereas Valtonen et al. (2006, road verges) and Hansen et al. (2021, grasslands) found evidence for changes in community composition and indications for homogenisation of plant communities as a result of invasion by lupine. We propose that the absence of significant effects on composition in our study, despite the relatively strong effects on diversity, may be attributed to the high proportion of species with low frequencies in our data set, which could influence the outcome of the multivariate analyses.

The strength of the effect of the invasive on resident plant species richness differed between study areas. As with other IAP, previous literature pointed to the strong context dependency of the effects of *L. polyphyllus* on plant communities (Pyšek et al. 2012, Tyler et al. 2015). For instance, Valtonen et al. (2006) found clear effects of the invader on species diversity, but Thiele et al. (2010) and Hansen et al. (2021) found smaller or context-dependent effects. Our study confirms context-dependence and indicates that it is because the magnitude of the effect of *L. polyphyllus* depends on the pre-existing community, with stronger negative effects on communities with higher proportions of poor competitive species relative to the proportions of strong and moderate competitors. While poor and very poor competitors were usually less common in plots with lupine, strong and moderate competitors showed neutral or positive response to lupine invasion. Differences in the composition of vascular plant communities is most likely the main explanation for stronger effects of lupine in the western study area. Competitive and moderately competitive species were more abundant in the eastern area before invasion (15 and 35 species, respectively), compared to the western area (11 competitive and 27 moderately competitive species). The number of poor and very poor competitors was the same in both areas (32 species), but these species were somewhat more abundant in the plots in the west.

The differences in community composition between study areas are most likely linked to the areas' dominating soil types and landscape structure, which both influence the land-use. Although high nature value road verges themselves in both areas are found on well-drained and fairly nutrient poor soils, clay-rich soils were more common in the studied verges in the east, and soils with sand and till more common in the west. In addition, nutrient rich soils and arable land are more common in the landscape as a whole in the east, and therefore competitive and nitrophilous species are more abundant in

Table 3. Results of the paired t-tests between plots with vs without lupine (lupine yes/lupine no) and between study areas (east/west). P-values in bold are significant at the 0.05 level or lower, and marginally significant effects are indicated in italics.

	Pairs	Average (cm)	t	df	p
Mean vegetation height	lupine yes/lupine No	53.83/11.28	13.71	23	**< 0.001**
Mean vegetation height	east/west	29.48/35.63	−1.79	23	*0.09*
Community-weighted vegetation height	lupine yes/lupine no	45.17/41.43	1.82	23	*0.08*
Mean litter depth	lupine yes/lupine no	1.63/1.51	0.72	23	0.48
Mean litter depth	east/west	1.16/1.98	−3.54	23	**0.002**

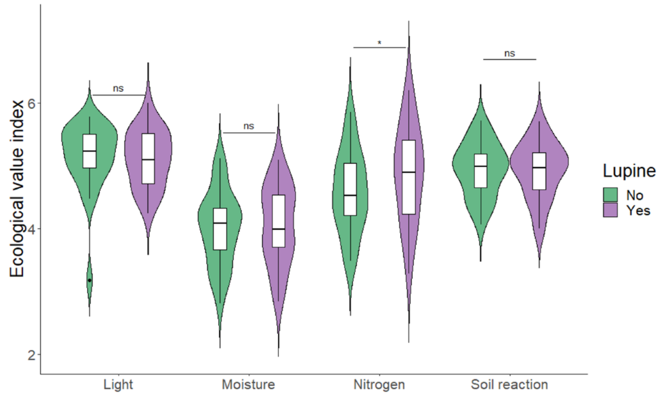

Figure 5. Differences in the mean community-weighted ecological indicator values between plots with (lupine: yes) and without (lupine: no) *L. polyphyllus*. The area surrounding the boxplots indicates the distribution shape of the data. Wider sections indicate a higher probability that the ecological indicator will take a value in that range.

the landscape and more frequently colonising and dispersing along road verges. Species pools at the regional scale were similar, i.e. all plant species except one (*Sagina revelierei*) recorded in the study occur in both areas according to distribution maps at the county level (https://artfakta.se). We did not expect any differences between study areas to be caused by road management, as all studied road verges had high conservation value (as identified by the Swedish Transport Administration), and therefore were subject to similar management, i.e. annual mowing late in the growing season.

Previously it has been shown that *L. polyphyllus* influences the biotic and abiotic characteristics of plant communities. Our results only partly agree with previous evidence. The mean vegetation height in plots dominated by lupine was higher than in plots without, corroborating the results of Valtonen et al. (2006) and Hansen et al. (2021). However, the community weighted vegetation height was not significantly different between plot types, indicating that the effect on community composition in terms of plant height-based functional traits was limited, and that the taller vegetation in lupine-dominated plots may partly be due to plastic response of the species. The overlap in species composition between the plots with and without lupine can also explain the lack of differences in the community weighted light, moisture, and soil reaction values between the type of plots. In the eastern, but not the western area, average light indicator value was higher and N indicator value lower in plots without lupine than in lupine dominated plots. This is expected if lupine makes the vegetation taller and fertilises the soil, thus benefitting the strong competitors. As mentioned, competitive nitrophilous species, having high N indicator value and low light value, were more abundant in the east. However, given that our study only included a total of 24 road verges, more studies that investigate how the community composition prior to invasion and other biotic and abiotic factors influence the effect of the garden lupine are needed.

Conclusions

This study has highlighted the significant threat posed by *L. polyphyllus* invasion to species richness and diversity in road verges. Based on the responses of plant species to *L. polyphyllus* and their affiliation to habitats in the surrounding landscape of the study areas, we can draw the conclusion that garden lupine invasion is likely to lead to reductions in both species richness and diversity of many plant communities within open and semi-open habitats. The effects will be most severe in communities with high proportions of low-competitive and nitrophobous species, such as dry, sparsely vegetated habitats, nutrient-poor ruderal (early successional) habitats, and low-productive semi-natural grasslands. The first two habitat types, in particular, constitute the majority of road verges of high nature value in Sweden (Lindqvist 2012). Low, sparse vegetation on well-drained soils is also a key microhabitat for invertebrates such as bees and wasps, carabid beetles, and grasshoppers. Also in other open habitats high plant species richness is usually associated with high number of short herbs (Hejcman et al. 2007).

In more productive grassland types characterized by medium tall, but still species-rich vegetation, a vast majority of the species seems to be negatively affected by an invasion of *L. polyphyllus*. Our study suggests that in road verges of high nature value, species with high, moderate, and low competition capacities can coexist, and that competitive species do not necessarily become dominant. *L. polyphyllus* invasion disturbs this equilibrium, diminishing the abundance of low-competitive species and potentially causing local extinctions. When *L. polyphyllus* is present species richness is reduced, both directly: by competition from the tall and dense lupine, and indirectly: by lupine favouring other competitive and nitrophilous species. While the direct effect of *L. polyphyllus* vanishes with its eradication, the indirect effect may persist until soil nutrient levels are restored. In order to minimise nitrogen enrichment, stands of *L. polyphyllus* should be removed as soon as possible after invasion.

Although only a few species of conservation concern were found in the studied road verges, many priority species for Swedish conservation have significant proportions of their known sites in transport infrastructure habitats (Helldin et al. 2015). To protect road verges of high nature value from the invasive *L. polyphyllus,* we recommend conducting vegetation surveys along road verges to identify vulnerable and prioritised plant communities, and regular monitoring of *L. polyphyllus* and other IAP to enable early eradication.

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Author contributions

Juliana Dániel-Ferreira: Data curation (lead); Formal analysis (lead); Visualization (lead); Writing - original draft (lead). **Tommy Lennartsson:** Conceptualization (equal); Methodology (equal); Writing - review and editing (equal). **Jörgen Wissman:** Conceptualization (equal); Investigation (equal); Methodology (equal); Writing - review and editing (supporting). **Carola Knudsen:** Investigation (equal); Methodology (equal). **Rolf Lutz Eckstein:** Conceptualization (equal); Investigation (equal); Methodology (equal); Writing - review and editing (equal)

Data availability statement

Data are available from the Swedish National Data Service (SND) at https://snd.se/en/catalogue/dataset/2023-259 (Dániel-Ferreira et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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