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RESEARCH ARTICLE

Undersowing oats with clovers supports pollinators and suppresses arable weeds without reducing yields

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Abstract

1. Sustainable food production requires agriculture to conserve biodiversity and facilitate ecosystem services to maintain productivity levels while reducing inputs detrimental to ecosystem functioning. Increasing within-field vegetation diversity by legume intercropping seems promising to facilitate cropping system multi-functionality. Effects of intercropping with legumes on biodiversity-mediated ecosystem services such as pollination or natural pest control are, however, not sufficiently understood.
2. Using 26 observation plots in a paired field design, we studied the effects of undersowing oats with a mixture of three annual clovers across different aspects of cropping system multi-functionality. We investigated 16 below- and above-ground ecosystem service indicators related to soil mineral nitrogen, arable weed control, pollination, disease and pest pressures, natural pest control and crop yield.
3. We found lower arable weed cover, higher flower cover and pollinator densities as well as decreased root-feeding nematode densities in intercropped observation plots compared with the non-intercropped controls. However, intercropping decreased spider activity densities and oat yield nitrogen content. Root diseases, pest damages, natural pest control and crop yield were not affected by intercropping.
4. The biomass of undersown clovers was positively related with the differences in flower cover and pollinator densities, and negatively related with the differences in arable weed cover between the intercropped and the control treatment.
5. *Synthesis and applications*: We demonstrate that undersowing annual clovers suppresses arable weeds and simultaneously support pollinators without reducing crop yields or taking land out of arable production. Undersown plant mixtures should, however, be tailored to support a wider spectrum of pollinators and benefit natural pest control to support a higher level of overall cropping system multi-functionality.

KEYWORDS
bumblebees, carabid beetles, cereals, ecological intensification, ecosystem services, ground-dwelling predators, intercropping, nematodes

1 | INTRODUCTION

Conserving biodiversity and ecosystem services in agricultural landscapes while maintaining sufficiently high yields is a great challenge towards sustainable food production (Bommarco et al., 2013; Kremen & Merenlender, 2018). Ecosystem services essential for crop production like pollination or natural pest control are driven by the diversity and densities of organisms delivering these services (Dainese et al., 2019). However, agricultural intensification and landscape simplification in the pursuit of higher crop yield levels are detrimental to the biodiversity of ecosystem service providers in agricultural landscapes (Landis, 2017; Seibold et al., 2019). The diversification of agricultural systems by increasing crop and non-crop plant diversity has been identified as a solution to avert ecosystem services erosion and increase sustainability of agricultural production (Bommarco et al., 2013; Isbell et al., 2017).

A widely promoted and implemented diversification strategy in agricultural landscapes is to establish special habitats that aim at supporting biodiversity, such as hedgerows, wildflower plantings or other semi-natural landscape elements (Boetzl et al., 2021). While creating such habitats comes at a direct cost of area taken out of crop production, the design of more diverse crop fields (Ekroos et al., 2016) has received less attention but may benefit wild biodiversity without reducing cropped area.

Diversification of cropping systems without a loss of cropped area can be realised by intercropping, that is the simultaneous cultivation of at least two different harvested or unharvested crops in the same field (Brooker et al., 2015). Intercropping is more common in subsistence and low-input agriculture of the tropics than in high-input agriculture, as it effectively increases the stability of total yield across years in low-input systems (Li et al., 2021). However, intercropping holds great potential for temperate high-input agriculture as, if crop complementarities are used efficiently, it can increase overall yields over longer time periods (Li et al., 2014, 2021). Intercropping can facilitate the access to limiting soil nutrients for one of the crops in nitrogen-limited systems (Li et al., 2014) and suppresses arable weeds in crops with poor weed competition ability (Gu et al., 2021; Verret et al., 2017). Especially intercropping cereals with nitrogen-fixing legumes as unharvested service crops incorporated to the soil as green manure and grown for ecosystem service delivery rather than direct economic benefit has been suggested to hold benefits for temperate agroecosystems (Brooker et al., 2015; Fletcher et al., 2016; Lagerquist et al., 2022). Legume intercropping in cereals further facilitates the availability of flower resources for pollinators and increases vegetation diversity which was shown to be beneficial for natural enemies and reduce pest densities (Wan et al., 2020).

Intercropping cereals or grain legumes with flowering legumes service crops has occasionally shown beneficial effects on functional biodiversity and ecosystem services, but usually at the cost of reduced yields. In soybean, undersown alfalfa promoted natural pest control by increasing natural enemy densities and delaying pest population growth but reduced yield by approximately 26% (Schmidt et al., 2007). In maize, intercropping with a flower mixture including legumes was shown to benefit overall arthropod biodiversity as well as pollinator assemblages, but reduced yields by 30%–50% due to competition in the early developmental stages of the maize (Norris et al., 2016, Norris et al., 2018). Besides a potential loss in yields, intercropping with service crops may also hold additional risks. Despite reductions in plant diseases due to intercropping of legumes in cereals in the majority of studies (Boudreau, 2013), legume intercrops can sometimes act as reservoirs for soil-borne pathogens of subsequently grown legumes (Šišić et al., 2018). Legume service crops can also increase the densities of detrimental root-feeding nematodes (Schmidt et al., 2017). However, undersowing clovers and other legumes in cereals has been shown to suppress arable weeds, promote nitrogen delivery and increase cereal yield in the subsequent year (Bergkvist et al., 2011; Lagerquist et al., 2022). Previously reported benefits as well as potential risks and drawbacks of intercropping systems are, however, mainly drawn from small-scale studies that focussed on single or a limited set of aspects of the respective cropping system. Especially temperate intercropping systems are so far insufficiently studied and conclusive, integrative assessments of multiple aspects of these systems are lacking.

Despite expectable benefits for ecosystem services, the uptake of intercropping with service crops in temperate agriculture is limited to date. The anticipation of lower crop yields and a potential build-up of pathogens and pests may affect the willingness of farmers to adopt intercropping with service crops. We aimed at investigating potential benefits of cereal legume intercropping on ecosystem services such as soil nitrogen provisioning, arable weed control, pollination and pest control as well as potential costs in the form of cereal yield loss or increased disease and pest pressures. For this, we used a study design with 26 paired intercropping and control plots on a semi-field scale with an intercropping system combining oats with an undersown mixture of annual clovers. We assessed multiple aspects of cropping system functionality using 16 ecosystem service indicators related to soil nitrogen, arable weed control, pollination, disease and pest pressures, pest control and crop yield. We hypothesised that undersown clovers (i) provide green fertilisation (thereby raising the amount of available nitrogen in the soil prior to sowing the subsequent crop), (ii) suppress arable weeds via competition, (iii) benefit pollinators via the provision of flower resources, (iv) benefit natural enemy assemblages above- and below-ground and enhance natural pest control and (v) lower cereal yields via competition. In addition, we assessed whether undersown clovers (vi) promote root diseases and root-feeding nematodes and whether (vii) observed differences in ecosystem service indicators scale with clover biomass.

2 | MATERIALS AND METHODS

2.1 | Study design

We selected 26 observation plots located within 13 oat fields on silt and clay dominated soils in the four counties Södermanland, Stockholm, Uppsala and Västmanland surrounding Lake Mälaren in south-central Sweden (Figure S1). Permission for this study was obtained from landowners. Of these fields, 10 were managed organically and three were managed conventionally, but no pesticides were used within observation plots in the conventional fields. The minimal distance between fields was 0.8 km (average between closest fields: 11.4 ± 2.7 km; measured from the closest field edges) and field sizes ranged from 1.8 to 47.8 ha (mean: 12.9 ± 3.9 ha; Table S1). In each field, we established two adjacent treatments: one treatment was sown with oats (*Avena sativa*; henceforth 'control') while the other was sown with oats (in the same density) and additionally undersown with a mixture of the three annual clover species *Trifolium incarnatum* (175 seeds * m^{-2}), *T. resupinatum* (150 seeds * m^{-2}) and *T. squarrosum* (175 seeds * m^{-2}; henceforth 'intercropped'; Figure 1). The spatial arrangement of the treatments was chosen at random. All fields were sown with oats between 7th and 29th April and harvested between 8th and 28th August 2020. The clover mixture was undersown on the same day or within 8 days from sowing oats. All sowings were conducted by the farmers using their machinery. Oat row spacing varied from 12.5 to 33 cm depending on the farmer, but it was always the same for the paired treatments and oat density did not differ significantly between them (Supporting Information I). Similarly, the clover mixture was either broadcast sown or sown in rows in between oats, depending on the machinery available. The two treatments within each field received identical management and fertilisation. The total area of intercropped and control treatments varied between fields from the intercropped treatment being equal to the size of the intercropped observation plot (50 * 20 m; see below) to encompassing almost the entire field apart from the control plot, with the size of the intercropped plots ranging from 0.1 to 8.3 ha (mean: 1.4 ± 0.6 ha).

2.2 | Data collection

Within each treatment, we established an observation plot of 50 m length and at least 20 m width (20–27 m, 22.9 ± 0.4 m; area: 1000–1350 m^2, 1146 ± 20 m^2) which was distanced from all field edges by at least 16 m (16–28 m, 21.4 ± 1.3 m; paired observation plots on the same field were always of equal size). Soil analysis (see methodology below) showed that the selected observation plots within each field did not differ significantly in soil pH, organic matter, clay, silt or sand contents prior to the experiment (Supporting Information I). All measurements within these observation plots were distanced from the plot edges by at least 5 m (i.e. at least 21 m from all field edges; Figure 1). In these observation plots, we recorded 16 indicators related to ecosystem service provision and crop production along three transects using soil samples (taken 1 year apart in the spring before and in the spring after the experiment), Braun-Blanquet squares, transect walks, pitfall traps, sentinel prey cards (aphid-cards) and plant biomass samples (detailed protocols for the assessment of these ecosystem service indicators can be found in Supporting Information I and Table S2). The ecosystem service indicators specifically related to soil mineral nitrogen, arable weed control (arable weed cover, arable weed biomass and granivorous carabid beetle density), pollination (flower cover and pollinator density), diseases and pest pressures (root disease severity, root-feeding nematode density and cereal leaf beetle damage), natural pest control (predatory nematode density, predatory carabid beetle density, staphylinid beetle density, spider density and predation rates) as well as crop yield (oat yield and oat yield nitrogen content). In addition, we recorded the biomass of undersown clovers in the intercropped observation plot (Supporting Information I).

2.3 | Statistical analyses

For all analyses, data were pooled on the observation plot level. Measurements that were taken before and after the experiment (all ecosystem service indicators obtained from soil samples, i.e. soil mineral nitrogen, root disease severity and root feeding and predatory nematode densities) were calculated as change by the treatment (i.e. the value before the treatment was regarded as baseline and subtracted from the value after the treatment).

All statistical analyses were performed in R 4.1.2 for Windows (R Development Core Team, 2021). We tested all ecosystem service indicators as response variables for an effect of our 'treatment' (factor, two levels: 'intercropped', 'control'). To account for the spatially nested design (i.e. two treatments per field), we used mixed-effects models or including 'field ID' as random intercept. The random intercept accounts for all field-level effects that are affecting both treatments equally (e.g. surrounding landscape, management or land-use history, but see also Supporting Information II). For responses with uneven sampling (i.e. uneven number of pitfall traps due to losses in the field), the sampling effort (i.e. days of pitfall trapping) was used as offset, transformed with the same link function as in the residual distributions used. We started with linear mixed-effects models ('lmer'; package LME4, version: 1.1-27.1, Bates et al. (2015)) using 'Gaussian' residual distribution and subsequently checked models for under- and overdispersion, zero inflation and suitability of chosen residual distributions using the packages DHARMA (version: 0.4.4, Hartig, 2022) and PERFORMANCE (version: 0.9.2, Lüdecke et al., 2021). If residuals did not follow a Gaussian distribution, we used generalised mixed-effects models ('glmmTMB'; package GLMMTMB, version: 1.1.2.9000, Brooks et al. (2017)) with negative binomial (with log link; for count data) or beta (with logit link; for proportions) residual distribution which improved fits. In cases where zero inflation was detected, it was accounted for in the 'glmmTMB' models. The commands, specifications and residual distributions used for each model are stated in the Supporting Information I (Table S3). All final models fulfilled their model assumptions.

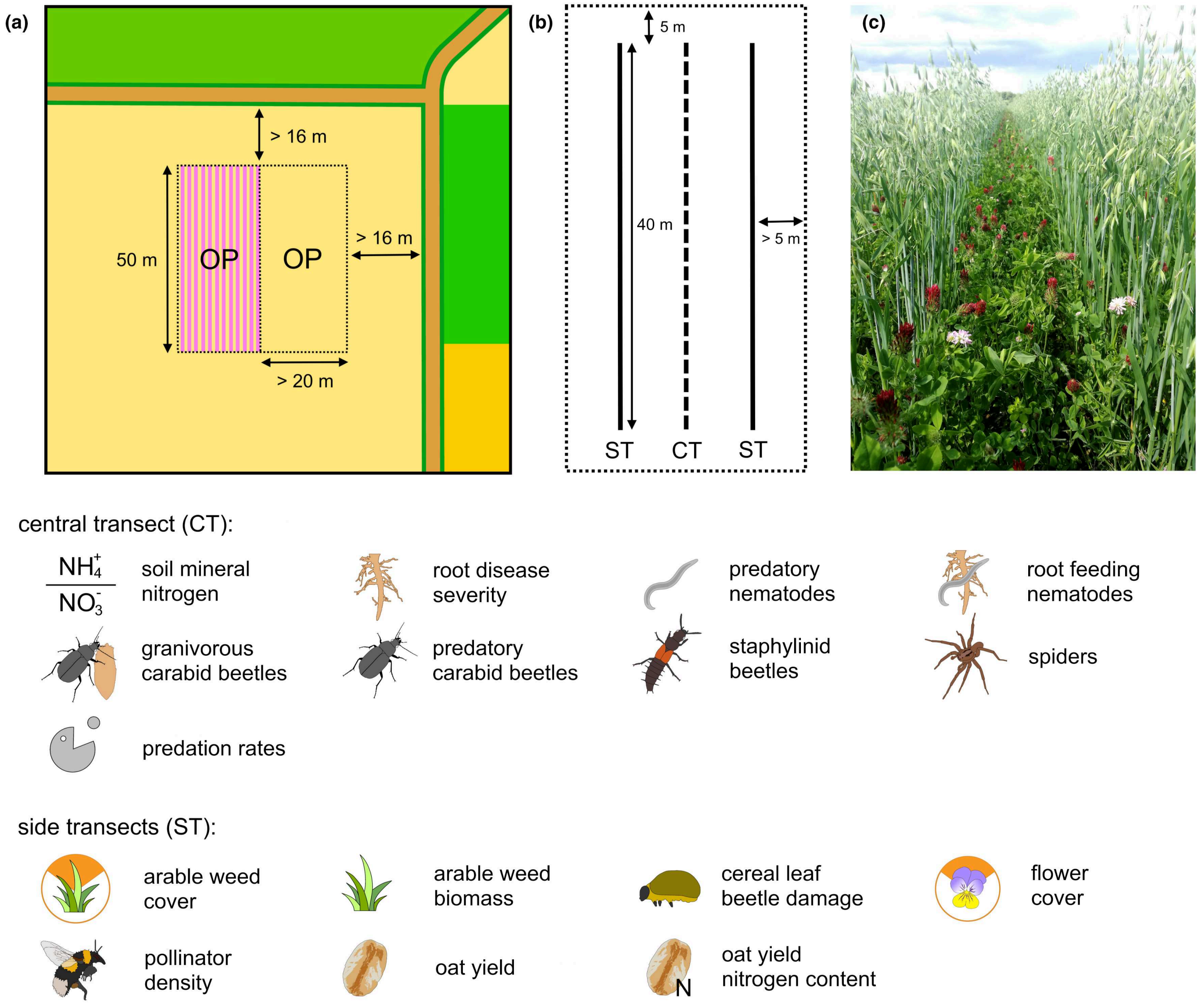

FIGURE 1 Schematic representation of the study design. (a) A study field located in a schematic, simplified landscape with two observation plots (OP) and distances. Colours: Yellow/light green: different field crops/agricultural land uses; purple: undersown annual clovers; brown: farm roads; dark green: margins. (b) Schematic of one of the two identical observation plots per field with distances as well as central transect (CT) and side transects (ST) along which several ecosystem service indicators were measured. (c) Undersown annual clovers in one of the oat fields (photo: Ola Lundin).

To assess whether the biomass of the undersown clovers determined the effects of intercropping on the ecosystem service indicators, we first calculated a difference for each ecosystem service indicator by subtracting the value of the control as baseline from the value of the intercropped observation plot. By this, the differences calculated for all indicators originating from soil samples that already represented a change between time points (after the treatment–baseline before the treatment) became a differences in changes. We used linear models with 'clover biomass' (continuous) in the intercropped observation plot as fixed effect as residuals followed a Gaussian distribution. Models were checked as described above and fulfilled their assumptions. In addition, we tested if some potentially influential factors related to nitrogen availability, nitrogen fertilisation and landscape context modified the intercropping effect (Supporting Information II, Tables S11 and S12). We verified that neither the proportion of the field covered by the intercropped treatment nor its total area were significantly correlated to any of the differences (Spearman correlations; $p > 0.05$). We also checked for nonlinear relationships between the differences in ecosystem service indicators and clover biomass using Spearman's rank correlations but results did overall not differ from those obtained using linear models (Table S10).

Model outputs were obtained using type 2 sums of squares Wald chi-square tests that divide the total variation equally among effects (command 'Anova' from library 'car', version: 3.0–12; Fox & Weisberg, 2019). Model coefficients were extracted using 'standardize_parameters (model, method = 'refit')' (package EFFECTSIZE, version: 0.5.0.10 (Ben-Shachar et al., 2020)).

3 | RESULTS

In the course of our assessment, we recorded 23 flowering plant species in addition to the sown clover species (Table S4), 169 pollinators (15 species; 67% *Bombus sp.*; Table S5), 2979 predominantly granivorous carabid beetles (14 species; Table S6), 5210 predominantly predatory carabid beetles (32 species; Table S6), 1321 staphylinid beetles (19 genera, not identified to species level), 5431 spiders (35 species; Table S7), 911 consumed aphids and 23.5 kg of harvested oat grains. Non-oat plant biomass was on average 67% higher in intercropped treatment than in the control.

3.1 | Soil mineral nitrogen

We expected intercropping oats with annual clovers to increase soil mineral nitrogen. However, the change in soil mineral nitrogen before sowing of the subsequent spring crop was not significantly affected by the treatment (Figure 2, Table 1 and Table S8).

3.2 | Weed control and pollination

As expected, intercropping decreased arable weed cover by 33% and tended to decrease arable weed biomass by 27% compared with the control (Figure 2, Table 1 and Table S8). The densities of predominantly granivorous carabid beetles did not differ significantly between the treatments (Figure 2, Table 1 and Table S8). Following our expectation, intercropping also increased flower cover on average by 479% (Figure 2, Table 1 and Table S8) and pollinator densities by 192% compared with the control (Figure 2, Table 1 and Table S8).

3.3 | Diseases and pests

We found no evidence for presumed negative effects of intercropping with clover service crops on root diseases or pests. Intercropping did not increase root disease severity compared to control (Figure 2, Table 1 and Table S8). In addition, root-feeding nematode densities decreased over time in both treatments but by 216% more in the intercropped treatment than in the control (Figure 2, Table 1 and Table S8). Cereal leaf beetle damage on oat plants did not differ significantly between treatments (Figure 2, Table 1 and Table S8).

3.4 | Natural pest control

We expected intercropping to benefit below- and above-ground natural enemies as well as pest control. However, intercropping did not significantly affect the change in predatory nematode densities (Figure 2, Table 1 and Table S8). Neither predominantly predatory carabid beetle nor staphylinid beetle densities differed significantly between the intercropped and the control treatment (Figure 2, Table 1 and Table S8). In contrast to our expectation, spider densities were on average 16%

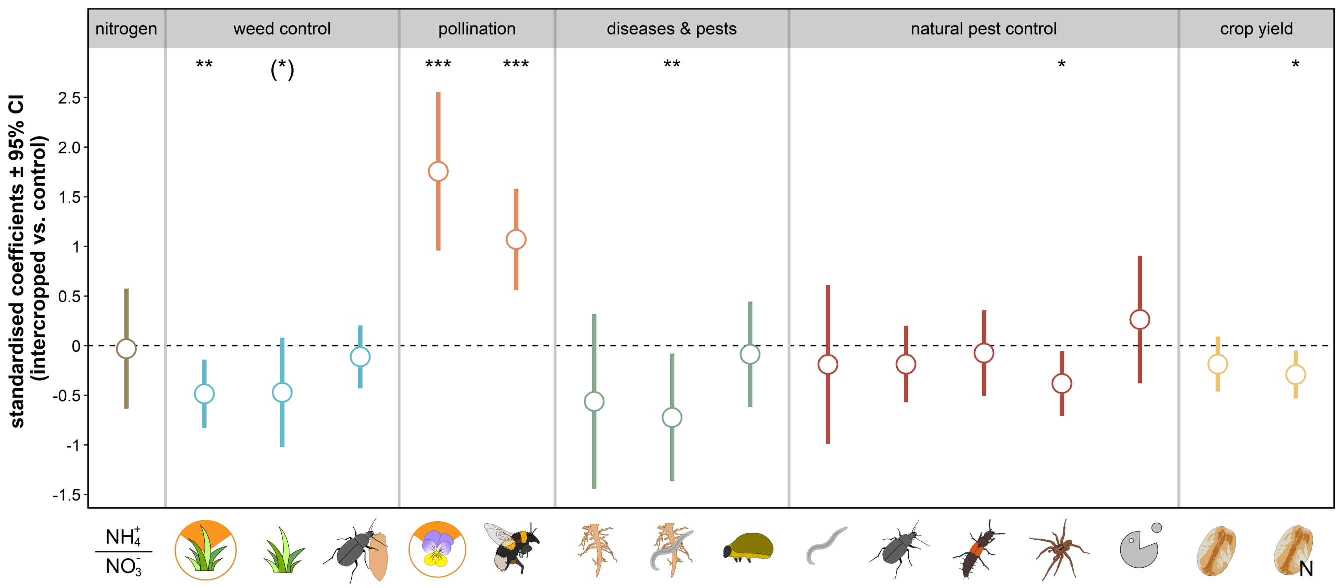

FIGURE 2 Model coefficients for the intercropped treatment against the control treatment for all ecosystem service indicators (positive values indicate the indicator is higher in the intercropped treatment than in the control treatment). Ecosystem service indicators (from left to right): soil mineral nitrogen, arable weed cover, arable weed biomass, granivorous carabid beetle density, flower cover, pollinator density, root disease severity, root feeding nematode density, cereal leaf beetle damage, predatory nematode density, predatory carabid beetle density, staphylinid beetle density, spider density, predation rate, oat yield and oat yield nitrogen content (for icons, see Figure 1). Coefficients for the differences between treatments (factor levels) are scaled to 1 standard deviation of the response in Gaussian models. In Poisson and negative binomial models, coefficients are on the log scale, in beta regression models, coefficients are on the logit scale (see Table S3 for model specifications). (*) indicates $p<0.1$; * indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.001$. For methods and statistics, see text and Table 1.

TABLE 1 Model results for the different ecosystem service indicators (separate models calculated for each indicator; see Table S3) comparing intercropped and control treatments. Coefficients for the differences between treatments (factor levels) are scaled to 1 standard deviation of the response in Gaussian models. In Poisson and negative binomial models, coefficients are on the log scale, in beta regression models, coefficients are on the logit scale (see Table S3 for model specifications). CI = confidence interval; observation plots = number of observation plots for which data was available for each ecosystem service indicator; Df = degree of freedom (numerator, denominator; corrected for nestedness on field); χ^2 = chi-square value obtained from Wald type II chi-square tests; p = p-value; R^2_m = marginal R^2; R^2_c = conditional R^2. 'difference' indicates that the indicator is calculated by subtracting a baseline measurement from the same observation plot before our treatment from the measurement taken after our treatment. Bold font indicates significant (p < 0.05) and marginally significant (p > 0.1) p-values; (*) indicates $p<0.1$; * indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.001$

Ecosystem service indicator	Model coefficient	95% CI	Observation plots	df	χ^2	p	R^2_m	R^2_c
Soil mineral nitrogen (difference)	−0.03	[−0.64; 0.57]	22	1, 18	0.01	0.916	<0.01	0.57
Arable weed cover	−0.49	[−0.83;-0.14]	22	1,18	7.60	**0.006****	0.08	0.87
Arable weed biomass	−0.47	[−1.02; 0.08]	24	1, 19	3.18	**0.074**(*)	0.06	0.60
Granivorous carabid beetle density	−0.11	[−0.43; 0.20]	24	1, 19	1.18	0.278	0.01	0.65
Flower cover	1.76	[0.96; 2.55]	24	1, 19	18.62	**<0.001*****	0.59	0.62
Pollinator density	1.07	[0.56; 1.58]	24	1, 19	17.01	**<0.001*****	0.22	0.70
Root disease severity (difference)	−0.56	[−1.44; 0.32]	22	1, 18	1.80	0.179	0.08	0.08
Root feeding nematode density (difference)	−0.72	[−1.37; −0.08]	22	1, 18	5.59	**0.018***	0.13	0.51
Cereal leaf beetle damage	−0.09	[−0.62; 0.44]	24	1, 19	0.10	0.749	0.01	0.39
Predatory nematode density (difference)	−0.19	[−0.99; 0.61]	22	1, 18	0.25	0.620	0.01	0.24
Predatory carabid beetle density	−0.19	[−0.57; 0.20]	24	1, 19	0.03	0.869	<0.01	0.64
Staphylinid beetle density	−0.07	[−0.51; 0.36]	24	1, 19	0.05	0.824	<0.01	0.43
Spider density	−0.38	[−0.71; −0.06]	24	1, 19	4.83	**0.028***	0.01	0.58
Predation rates	0.26	[−0.38; 0.91]	24	1, 19	0.79	0.375	0.02	0.46
Oat yield	−0.19	[−0.46; 0.09]	24	1, 19	1.97	0.161	0.01	0.90
Oat yield nitrogen content	−0.29	[−0.53; −0.05]	24	1, 19	6.31	**0.012***	0.02	0.92

lower in intercropped treatment compared with the control, although this decrease became less pronounced and not significant if analyses were limited to adult spiders (Supporting Information I, Figure 2, Table 1 and Table S8). Predation rates also did not differ significantly between the treatments (Figure 2, Table 1 and Table S8).

3.5 | Crop yield

We expected the addition of annual clovers to lower oat yield due to competition. However, oat yield did not differ significantly between the treatments (Figure 2, Table 1 and Table S8) but the nitrogen content in the grains was on average 4% lower in the intercropped treatment compared with the control (Figure 2, Table 1 and Table S8).

3.6 | Effects of clover biomass in intercropped treatments

The observed differences between the intercropped treatment and the control for the investigated ecosystem service indicators raised the question whether the impact of intercropping was modulated by the biomass of undersown clovers. Though sown at the same densities at all sites, the clover mixtures varied in establishing success and growth between fields (mean dry biomass: 991 ± 227; median: 566; range: 175–2950; kg * ha^{-1}). This gave us the opportunity to relate our ecosystem service indicators to clover biomass.

Clover biomass was positively related with the differences in flower cover (F = 47.92; $p<0.001$ ***; Table S9) and pollinator density (F = 15.49; $p<0.001$ ***; Table S9) and negatively related with the difference in arable weed cover (F = 6.54; p = 0.015 *; Table S9) between the intercropped treatment and the control (Figure 3a–c, Table S9). The differences between intercropped and control for all other ecosystem service indicators were not significantly related to clover biomass (Figure S2, Table S9).

4 | DISCUSSION

Intercropping oats with annual clovers suppressed arable weeds and benefitted pollinators without increasing disease or pest pressures or being detrimental to natural weed control potential, natural pest control potential or crop yields. However, undersown clovers failed to increase soil mineral nitrogen or facilitate beneficial natural

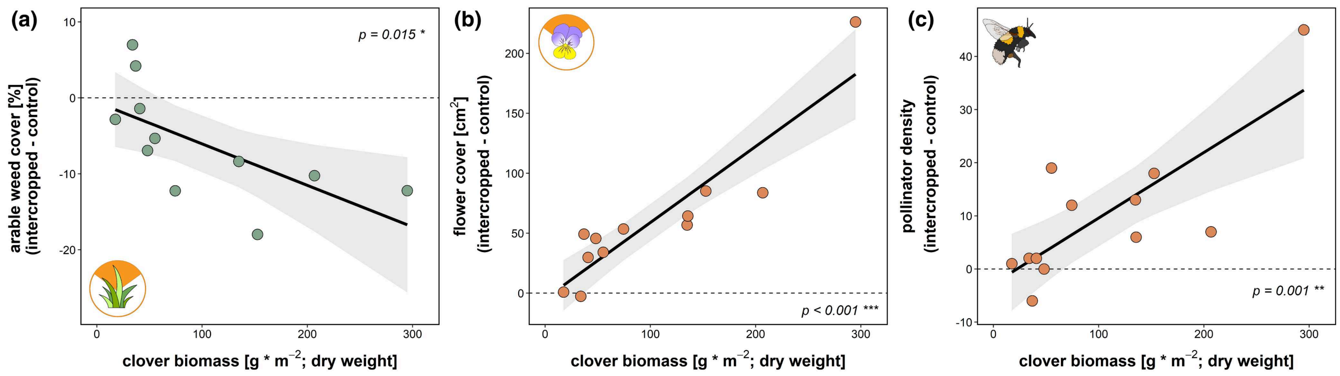

FIGURE 3 Differences in (a) arable weed cover, (b) flower cover and (c) pollinator density between intercropped and control treatments in relation to clover biomass in the intercropped treatment. Prediction obtained from the linear models. Solid lines represent predictions, grey shaded areas 95% confidence intervals. Colours correspond to those used in Figure 1.

pest control or natural enemy densities beyond the level found in the control. While our intercropping system still holds potential for improvement to achieve higher levels of cropping system multifunctionality, it shows clear improvements compared with single crop cultivation.

The change in soil mineral nitrogen between the spring before our treatment and the following spring was similar in the intercropped treatment and the control despite expected beneficial effects of undersown clovers for mineral nitrogen stocks based on previous studies (Bergkvist et al., 2011; Cadoux et al., 2015). However, expected changes in soil nitrogen are small and depend on clover biomass as well as on the timing of the termination of the clover service crop. Our service crops were terminated in autumn by physiological age, tillage or frost, which could lead to nitrogen losses over the winter. A continuation of the intercropping with frost tolerant clover species as cover crop might reduce nitrogen losses and benefit the subsequent crop (Cadoux et al., 2015; Lagerquist et al., 2022).

Our intercropping treatment reduced arable weed cover by 33% and tended to reduce arable weed biomass by 27%, which is in accordance to results obtained for service crops predominantly in maize and wheat (Petit et al., 2018; Verret et al., 2017). The observed weed control in our intercropped treatment was likely driven by competition for nutrients, water and light between the annual clovers and the arable weeds (Liebman & Dyck, 1993; Verret et al., 2017). The negative relation found between weed cover difference between the treatments and clover biomass supports this assumption. Our results indicate that management for higher clover biomass increases arable weed control without any yield penalty within the range evaluated. Intercropping oats or other cereals with annual clovers may thus provide direct economic benefits via reducing the need for mechanical weed control in organic farming and herbicide use in conventional farming. The densities of granivorous carabid beetles are usually positively related to arable weed cover via the availability of weed seeds as prey (Carbonne et al., 2022). We found densities of granivorous carabids not affected by intercropping despite negative effects on arable weed cover and a negative trend on arable weed biomass. This indicates that undersowing annual clovers, at least in the short term, does not disrupt natural weed control potential by granivorous carabids.

Intercropping increased overall flower availability substantially, even with undersown annual clovers suppressing arable weeds of which many also provided floral resources. The vast majority of available flowers were, however, directly provided by the undersown clover plants (Table S4). Generally, higher flower cover also leads to higher local pollinator densities (Potts et al., 2003; Steffan-Dewenter & Tscharntke, 2001) which is in line with our results. A local increase in flower plantings, however, also increases bee populations on the landscape level (Kleijn et al., 2018; Rundlöf et al., 2014). Especially in landscapes with temporally or permanently scarce floral resources, a mixture of undersown clovers with a long flowering time can provide alternative flower resources to stabilise pollinator populations without sacrificing cropland for separated wildflower plantings. Most of the pollinators recorded in our field experiment were long-tongued (mainly bumblebees) which is not surprising due to clover flower morphology (Vleugels et al., 2019). Pollinator diversity is usually linked to flower resource diversity (Albrecht et al., 2020; Steffan-Dewenter & Tscharntke, 2001) and increasing pollinator diversity is positively related to pollination (Fründ et al., 2013). Thus, undersown plant mixtures could be optimised to support a higher overall pollinator diversity and functional richness. As the beneficial effect on flower cover and pollinator densities both scaled positively with clover biomass without any obvious detrimental effects on oat yield, higher clover biomass would be favourable for supporting long-tongued pollinators.

Overall, we found no increase in disease or pest pressures due to the undersown clovers. Despite previous indications that legume cultivation can favour root pathogens and thus harm subsequent legume crops (Šišić et al., 2018), we found no increase in root disease severity in following legume crops grown on soil taken from intercropped treatment compared with the control. However, longer timeframes for plant disease monitoring may be needed as legume intercropping implemented consecutively across years might lead to an accumulation of pathogens. In contrast to previous results for legume service crops, our legume intercropping did also not increase but instead decreased root-feeding nematode densities compared with the control (Schmidt et al., 2017). A possible reason for this is that the suppression of arable weeds in the intercropped treatment reduced associated root-feeding nematode densities (Thomas et al., 2005). Cereal leaf beetle damage was similar in both treatments, despite a negative effect of intercropping on spider densities. This decrease in spider densities was, however, small and cereal leaf beetle damage levels overall low which could have disguised possible effects. Overall, the very low pest densities in our fields (Supporting Information I) may have camouflaged potential effects of intercropping.

Increasing vegetation diversity in crop fields has been shown to benefit natural enemies and thereby increase natural pest control (Wan et al., 2020) via increased resource availability and improved microclimatic conditions (Diehl et al., 2012). In contrast, our intercropping treatment failed to facilitate natural pest control: neither above- nor below-ground natural enemies benefitted from intercropping oats with annual clovers and spider densities even decreased. Consequentially, predation rates on the ground level, which are linked to above-ground natural enemy densities (Boetzl et al., 2019), were also not increased by intercropping. However, intercropping did also not disrupt natural pest control as occasionally reported (Gontijo et al., 2018) with no effects on pest damage or crop yields found. The assessed ground-dwelling natural enemies could respond slower to alterations as they are less mobile and have smaller action radii than social bee pollinators that are recruited from nesting sites in the surrounding landscape (Kleijn et al., 2018). Longer timeframes might thus be needed to observe effects of undersown clovers on natural enemy densities. It is also plausible that undersowing three closely related and functionally similar clover species did not improve life conditions for natural enemies in oat fields substantially. Increasing the functional diversity of undersown service crop mixtures may thus benefit natural enemy populations if suitable plant species and traits are identified (Moreira et al., 2016).

Overall, undersown clovers did not significantly decrease oat yields and this effect was also not affected by the biomass of undersown clovers (within the range evaluated). Undersowing legume service crops and especially clovers in additive intercropping systems was previously shown to not decrease cereal yields in most cases (Iverson et al., 2014), but specifically for oats data are scarce (Verret et al., 2017). Our intercropping with annual clovers did, however, reduce the nitrogen content of the oat yield by 0.7 g per kg oat grains (approximately 4%). It has been reported that legume intercropping often increases cereal grain nitrogen content (Bedoussac et al., 2015), but the present results indicate late season competition between oats and undersown clovers. The undersown clovers had a higher proportion of their growth later in the season than the oats and clover biomass accumulated mainly during grain filling; thus, the fixed nitrogen was probably not available in time to benefit the oats (Fletcher et al., 2016). In addition, clovers cannot be expected to be fully nitrogen self-sufficient. When established as undersown service crop in oats, only about 75% of the required nitrogen is fixed from the air (Lagerquist et al., 2022). However, the observed decrease is small and in contrast to other cereals such as wheat or barley used for baking and malting, nitrogen content in oat grain is usually not of economic interest (Christoffersson et al., 2021).

The fear of yield loss and presumed higher management costs are so far limiting the implementation of intercropping in temperate agriculture despite known benefits (Brooker et al., 2015). We show here that benefits can be achieved without reduced crop yields and with limited additional costs of machinery as the regular sowing machine was used for sowing the clover mixture. Ideally, however, undersown plant mixtures should also facilitate further ecosystem services such as nitrogen delivery and natural pest control and thus help reduce fertiliser and pesticide inputs and yield losses to pests to compensate for the cost of seeds and labour. Undersown mixtures thus need to be improved to simultaneously benefit a wider array of ecosystem services. One potentially important factor in this respect could be the functional difference between undersown plant species—we used closely related clovers that have similar ecological niches, functions and traits and presumably support similar insect species. Increasing phylogenetic differences could create a more diverse vegetation structure and offer of floral resources and traits and increase ecological contrast within the mixtures and between the mixtures and the main crop. Ecological contrast has been shown to benefit many insects (Marja et al., 2019; Scheper et al., 2013) and an increased plant diversity is commonly found to be beneficial for many ecosystem service providers such as arable weed controlling carabid beetles (Carbonne et al., 2022), pollinators (Albrecht et al., 2020; Scheper et al., 2013; Steffan-Dewenter & Tscharntke, 2001) and natural enemies (Wan et al., 2020). However, trade-offs and limitations of such diversified undersown mixtures need to be assessed carefully to prevent boosting pathogens, pests or competition with the main crop and thereby hampering crop yields. Future research should further assess potential factors that might increase the positive impact of intercropping with undersown legumes to maximise its benefits.

We showed that intercropping of cereals with legume service crops holds great potential for the transformation of agricultural landscapes to support higher levels of functional biodiversity and higher ecosystem service potentials without taking cropland out of production. Despite remaining limitations in terms of increasing natural pest control, the benefits are striking while substantial detriments are absent.

AUTHOR CONTRIBUTIONS

Ola Lundin, Anna Douhan Sundahl, Göran Bergkvist, Maria Viketoft and Hanna Friberg designed the study, Anna Douhan Sundahl and Ola Lundin conducted the field work, Anna Douhan Sundahl, Maria Viketoft and Hanna Friberg analysed the samples and Fabian A. Boetzl analysed the data and wrote the first draft of the manuscript. All authors interpreted the results, revised the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data used in this study are available from the Swedish University of Agricultural Sciences Archive: https://hdl.handle.net/20.500.12703/4002. (Boetzl et al., 2022).

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SUPPORTING INFORMATION

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

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