

# Ant community composition and functional traits in newly established grasslands within agricultural landscapes

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## Abstract

1. Ongoing intensification and fragmentation of European agricultural landscapes dramatically reduce biodiversity and associated functions. To sustain ecosystem services such as ant mediated pest control, the enhancement of perennial non-crop areas holds great potential. 2. To study the potential of newly established grasslands to enhance ant diversity and associated functions, we used hand collection data to investigate differences in ant community composition (a) between cereal crops, old grasslands, and new grassland transects of three years age; (b) depending on ant functional traits; and linked to (c) natural pest control services quantified through predation experiments. 3. Ant species richness did not significantly differ between new and old grasslands, but was significantly higher in grasslands compared to cereal crops. Contrary, ant community composition of new grasslands was more similar to cereal crops and distinct from the species-pool of old grasslands. The functional trait space covered by the ant communities overlapped between old and new grasslands but was extended in the old grasslands. Pest control did not differ significantly between habitat types, and therefore could not be linked to the prevalence of functional traits related to biocontrol services in new grasslands. 4. Our findings show trends of convergence between old and new grasslands, but also indicate that enhancing ant diversity through newly established grasslands takes longer than three years to provide comparable biodiversity and functions. 5. Synthesis and applications Newly established grasslands can increase ant species richness, abundance, and pest control in agroecosystems. However, three years after establishment, new grasslands were still dominated by common agrobiont ant species and lacked habitat specialists present in old grasslands, who require a constant supply of food resources and long colonization times. New grasslands represent a promising measure for enhancing agricultural landscapes but must be preserved in the longer term to sustain biodiversity and associated ecosystem services.

## 5. Synthesis and applications

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## Keywords

agricultural biodiversity, ant community composition, functional traits, natural pest control, ecosystem services, newly established grasslands

# 1 Introduction

European countries are spatially dominated by agricultural landscapes (Kleijn et al., 2012), yet the ongoing intensification of their management dramatically reduces biodiversity (Brooks et al., 2012; Cardoso et al., 2020). Even though species diversity and specific ecological traits are well known as key promoters of ecosystem functioning (Borer, Grace, Harpole, MacDougall, & Seabloom, 2017), ubiquitous ecosystem engineers, such as ants (Sanders & van Veen, 2011), are threatened by destruction (Hendrickx et al., 2007) and fragmentation of remaining semi-natural habitats interspersed between arable land (Ewers & Didham, 2006). In order to mitigate severe effects on the maintenance of ecosystem services provided by ants, such as biological pest control (Östman, Bengtsson, & Ekborn, 2001; Tscharnatke et al., 2012), biodiversity restoration in modern cultivated landscapes holds great potential (Tscharnatke, Thies, Kruess, Steffan-Dewenter, & Klein, 2005; Ekroos, Olsson, Rundlöf, Wätzold, & Smith, 2014). Extensively managed grassland ecosystems are among the most species-rich habitats in Northern and Central Europe (Krauss, Steffan-Dewenter, & Tscharnatke, 2003) and paramount for the diversity of ants in temperate regions (Seifert, 2018). These important semi-natural environments are disappearing rapidly from European agricultural landscapes due to abandonment, afforestation and conversion to residential areas (Dengler, Janišová, Török, & Wellstein, 2014; Valkó et al., 2018). Set-aside land and other remnants of semi-natural habitats enhance the edge density in agricultural landscapes, which fosters the diversity and abundance of ground-dwelling predators like ants (Haaland, Naisbit, & Bersier, 2011; Martin et al., 2019) and correspondingly the success of biological pest control in adjacent farmland (Gardiner et al., 2009). However, yield-enhancing ecosystem services rely heavily on the ability of predator species to disperse into the agricultural matrix (Kohler, Verhulst, Van Klink, & Kleijn, 2008). The enhancement of perennial non-crop areas through newly established grassland strips, likely provides refuge habitat not only for common agrobiont species, but also for more specialized species, if they persist in the long-term (Dauber & Wolters, 2005).

Ants are eu-social insects and important consumers of herbivorous insects, which makes them a key taxon for ecosystem functioning of temperate grasslands (Wills & Landis, 2018). Many ant species can organize mass-recruitment if sufficient food sources are available (Seifert, 2018) and this spatial allocation of predatory workers enables ant colonies to respond effectively to a dynamic and heterogeneous density of prey in their environment (Way & Khoo, 1992). Yet, the importance of ants as consumers and ecosystem engineers is often underappreciated (Wills & Landis, 2018), even though they are the numerically dominant invertebrates in certain agricultural landscapes. Offenberg (2015) showed that the efficiency of ant-mediated biocontrol is comparable to chemical pesticides, which designates ants as a relevant target group towards the development of sustainable management practices of agroecosystems.

Similar to most other grassland taxa, ants are highly responsive to human impact, such as land use change (Dahms et al. 2005; Dauber et al. 2006). A recent study highlights that ant species richness, as well as functional diversity of ant communities, decreases with increasing land-use intensity in terms of mowing and grazing of grasslands (Heuss, Grevé, Schäfer, Busch, & Feldhaar, 2019). However, to maintain ant biodiversity and their role as biocontrol agents, not only the underlying mechanisms leading to the aforementioned decreases have to be elucidated, but also how habitat restoration, in terms of newly established grasslands, may affect ant communities. Along this line, it is essential to consider that colonies of all ant species in temperate regions require multiple years to establish, grow and reproduce (Dauber & Wolters, 2005; Seifert, 2018). Hence, in an agricultural landscape long-term set-aside area and permanent grassland interspersed between arable field crops is required to keep or bring ants back as ecosystem engineers.

Understanding the ecological function of a species in a particular habitat requires knowledge of species-specific traits, their dependence on environmental factors and ecological niches (Cadotte, Carscadden, & Mirotchnick, 2011; Gagic et al., 2015). Moreover, functional traits of ants, such as colony size (number of individuals), predation on pest insects (proportion of animal based resources in ant diet) and recruitment behaviour are closely linked to the biological control services they may provide (Perović et al., 2018). Moreover, ant functional traits are linked to species-specific responses of ants to habitat alteration and management intensity of agroecosystems (Ekroos, Rundlöf, & Smith, 2013).

This study aimed to document the development of ant community composition and functional diversity within newly established grassland strips of three years age and adjacent cereal crops. The results were compared to reference plots in traditionally used old grasslands, and control plots situated in the surrounding crops. Pest control potential was quantified in all habitats through predation experiments with sticky-cards using fruit flies as baits. Further, vegetation cover of all habitats was recorded, as a high and dense vegetation supports a higher supply of food resources (Kruess & Tscharntke, 2002; Siemann, 1998) and thus might affect the dependency of predatory arthropods such as ants to feed on experimentally exposed fruit flies.

At the start, newly established grasslands are assumed to provide habitats primarily for common agrobiont ant species (Dauber & Wolters, 2005) but they may also be colonized by habitat specialists if they persist in the long term and offer diverse ecological niches. Further, social insects such as ant colonies have high and continuous nutrient requirements and should therefore play a key role as biocontrol agents in agroecosystems. In account of that, the following research questions were addressed:

- 1) How do species richness and community composition of ants compare between newly established grasslands, old grasslands and surrounding cereal crops?
- 2) Are newly established grasslands able to develop the functional trait space covered by ant communities in old grasslands, and further the prevalence of traits related to biocontrol services?
- 3) How do the investigated habitat types and vegetation cover affect provided pest control services, and can predation efficacy be related to the aboveground activity of ants?

## 2 Methods

### 2.1 Study area

All field experiments were performed within the framework of the research project “REGRASS” (*Re-establishing grassland-strips to promote biodiversity and ecosystem services*) that was located near the villages Elsbach (48°15′08.3″N 16°02′56.9″E) and Ollern (48°16′02.5″N 16°05′07.9″E) in the area of Sieghartskirchen, Lower Austria (mean annual air temperature: 12.4 °C, mean annual precipitation: 628 mm; Kreuth 2019). The region is characterised by small-scale but intensively managed agricultural land, along the foothills of the Wienerwald forests. Within the study region (Elsbach, Ollern) five crop fields were selected, adjacent to extensively managed, semi-natural pasture (old grasslands; OG). In each of which three different transects directly adjacent to the old grasslands were established (see Fig. 1): newly established grasslands (NG), a transect within cereal fields ten meters next (CN) to new grasslands and within cereal fields in far (CF) distance of >80 meters to new grasslands. Each transect contained six sampling plots at a regular distance of 35 meters, making up 15 transects comprising 90 sampling plots in total. The first sampling plot of each transect was located in the old grasslands. Grassland ant species, as well as biocontrol potential were investigated over a period of two months between 8<sup>th</sup> of April to 7<sup>th</sup> of June 2019.

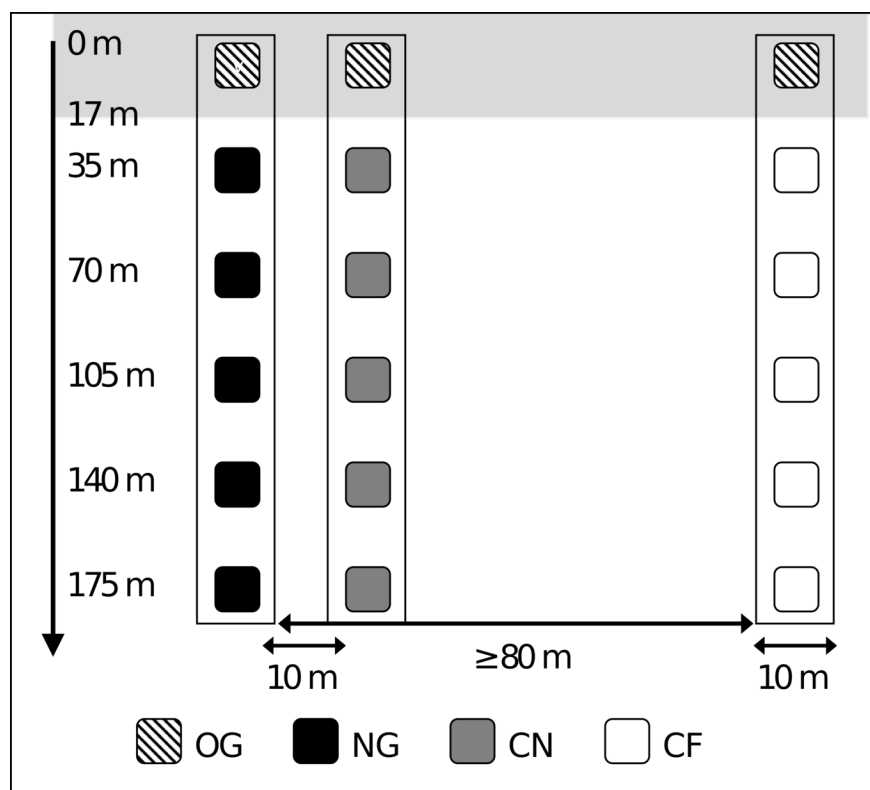


Figure 1: Schematic sketch of ant observation sites in each study location. Each of the three transects contained six sampling plots at increasing distance to the adjacent old grassland (semi-natural habitat remnant; grey area on top). Hatched squares (**OG**) = first sampling plot of each transect in old grassland (reference plots); black squares (**NG**) = sampling plots within newly established grassland; grey squares (**CN**) = sampling plots in adjacent cereal field near to NG; white squares (**CF**) = sampling plots in control cereal field far from NG.

The new grasslands had been established in August 2016 in five winter cereal fields directly adjacent to selected areas of old grassland. In order to mimic the native plant community of the old grasslands, the new grasslands were sown with a variety of seeds from 54 different plant species native to the region (30% grasses, 55% herbaceous plants and 15% legumes). The new grasslands were mowed once every year in late summer and the old grasslands in late June. The use of tillage in the cereal field transects was avoided by the farmers during the sampling period between April and June, but otherwise field management (such as the use of pesticides) continued here.

## 2.2 Data recording/sampling

Recording of ant activity and species diversity was done by hand collecting of worker ants with fine tweezers. Hand collection has been discussed as the most efficient method for sampling ants (Gotelli et al. 2011). It generates results comparable to those from pitfall traps (Andersen, 1991; Sanders, Barton, & Gordon, 2001), and is not biased in favour of behaviourally dominant species that monopolize food-resources (Andersen, 1997), which may occur when using bait traps. Over the sampling period a total of three consecutive survey runs on each of the 90 sampling plots was performed, with 14 to 21 days between each run. For statistical evaluation, the results of all three runs were aggregated for each transect/habitat. On each sampling plot two 1x1 m sized quadrants around the center were searched for foraging worker ants for four minutes each per run. Worker ants active around nests were also sampled and the total aboveground nest activity (in ants

per four minutes) estimated. Prior to the hand sampling also the vegetation cover (0-100 % of soil covered) was estimated in a radius of 2 m around the plot center. All collected individuals were preserved in 70 % ethanol and later identified to species level according to Seifert (2018) using a stereo-microscope at 10-fold magnification.

Measuring of biocontrol potential was done with sticky-card experiments, using adult *Drosophila melanogaster* (Meigen) flies as baits (Lys, 1995). Over the sampling period a total of four consecutive survey runs on each sampling plot was performed. For statistical evaluation, the results of all four runs were aggregated for each sampling plot/habitat. For each sticky-card, thirty flies were glued to the upper side of a 6x8 cm cardboard, which had a plastic underlay (to protect the card from soil moisture), and fixed to the ground with a long nail. Flies were glued to the cardboard with well-diluted fish-glue enabling ground-dwelling predatory arthropods to remove the prey which guarantees successful predation (Lys, 1995). Each cardboard was covered by an enclosure with an appropriate mesh size (1x1 cm) preventing the access of rodents and birds, thus enabling effectively to measure predation on flies by arthropods (Hulme, 1996). Two cardboards were placed on each sampling plot per survey and exposed to predatory arthropods for two and a half to 3 hours. Afterwards, predation rates (number of destroyed/killed flies) and the estimated vegetation cover of the sampling plots (0-100 % of surface covered) were recorded directly in the field.

## 2.3 Ant traits

Life history traits of all ant species encountered were taken from Seifert (2017) and (2018) and Arnan et al. (2017). All trait data and a detailed description of trait categories are provided in the appendix: see Tables S1 and S2. The subsequent statistical analysis determined the overall functional trait space covered by the ant communities and examined in detail a selection of traits which are closely linked to biocontrol services.

## 2.4 Statistical Analyses

All statistical analyses were conducted in the statistical programming environment R (Version 3.6.2, R Core Team 2019). Cumulated ant species richness (ant species in transects pooled per habitat type) was compared across habitat types according to a Monte-Carlo randomization test procedure (Manly, 2006) using the “rich”-package (Rossi, 2011). To investigate how species replacement (turnover) and species loss (nestedness) account for the variation in species composition (beta diversity), the total dissimilarity expressed as Sørensen index ( $\beta$ SOR) across the four habitats, as well as its respective turnover ( $\beta$ SIM) and nestedness ( $\beta$ SNE) components, were calculated using the package “betapart” (Baselga & Orme, 2012).

In order to study the influence of habitat type on ant species composition of the transects, a constrained ordination analysis was performed. A dummy species with an abundance of one in all samples was added to the presence/absence data, to deal with low numbers of species per transect (Clarke, Somerfield, & Chapman, 2006). Based on this dataset a Sørensen dissimilarity matrix was created using the package “vegan” (Oksanen et al., 2018). Subsequently, a canonical analysis of principal coordinates with two axes on the Sørensen dissimilarity matrix was performed, with habitat type as a constraint variable. Differences between the habitat types were tested for significance with a PERMANOVA using “adonis” function, where the habitat type served as fixed factor and the study region (Elsbach, Ollern) as random factor.

A principal component analysis of the species-trait data was performed using the package “FactoMineR” (Lê, Josse, & Husson, 2008) and the first two principal coordinates of each species plotted in a two-dimensional diagram. In order to display the functional trait space covered by ants in the different study habitats, a convex hull (polygon) was drawn around the respective species communities. Differences between the habitat types were tested for significance with a PERMANOVA based on an Euclidean distance matrix of the species trait data.

Community weighted mean (CWM) values of selected ant species traits were calculated using the “FD”-package (Laliberté & Legendre, 2010; Laliberté, Legendre, & Shipley, 2014). The calculated CWM values

refer to the average of species trait values at each sampling transect weighed by the relative species abundance (Lavorel et al., 2008; Ricotta & Moretti, 2011). As the observed abundance of foraging workers showed high fluctuations caused by e.g. life cycle stage of ant colonies (Seifert, 2018), the analysis was based on a pseudo-abundance matrix, which refers to the presence of the respective species on the number of runs (0-3) on each plot pooled per transect. Cereal field habitats were excluded from the analysis, as a reliable calculation of CWM requires at least three species, which was not given for the majority of transects of these habitats. The analysis focussed on three distinct traits: *proportion of animal-based resources* in ant diet (*Zoophaga* ; food resources acquired via predation or scavenging, see Table S1), *recruitment behaviour* of workers (*FS* ; foraging strategy) and *colony size* (*CS* ; number of individuals). In order to increase the suitability of the CWM values for linear models and to attain normal distribution, logit transformation was applied for the CWM values of the traits *Zoophaga* and *FS* and log transformation for the trait *CS* . Using the CWM values of the selected traits as response variable, the habitat type (OG, NG) as predictor variable and study region as random factor three generalized linear mixed models (GLMM) were created using the package “lmerTest” (Kuznetsova, Brockhoff, Christensen, & Jensen, 2019), which computes p-values via the Satterthwaite approximation. To access p-values for the comparison among fixed factor levels, Tukey’s post-hoc tests were conducted using the package “multcomp” (Hothorn, Bretz, & Westfall, 2008). The same approach was applied for the subsequent models.

To investigate the effect of habitat type and mean vegetation cover (0-100 % of sampling plot surface covered) on predation intensity on sticky cards, a predation rate (0-1) was calculated based on the number of eaten flies per sampling plot summed across all four survey runs (n per 240 flies in total; 30 flies x 2 cards per plot x 4 runs). Subsequently, a GLMM was created, with the predation rate per sampling plot as response variable, habitat type and logit transformed mean vegetation cover of the sampling plot as fixed factors and study region as random factor. Furthermore, the same GLMM approach was used to test whether logit transformed mean vegetation cover showed significant differences between habitat types. Fixed effect structures and GLMMs were compared using the packages “multcomp” and “MuMIn” (Bartoń, 2019).

To study the effect of habitat type on aboveground ant activity, the number of observed worker ants per sampling plot was summed across all three survey runs and transformed with Tukey’s Ladder of Powers, in order to attain normally distributed values, using the package “rcompanion” (Magnificio 2019). Subsequently, a GLMM was created with Tukey transformed number of observed workers per sampling plot as response variable, habitat type and logit transformed mean vegetation cover of the sampling plot as predictor variables and study region as random factor. Further, the correlation of predation rate on sticky cards and Tukey transformed aboveground ant activity was tested with a GLMM with study region as random factor, again. Marginal and conditional  $R^2$  values ( $R^2_m/R^2_c$ ) of the GLMM were calculated using the package “MuMIn”.

## 3 Results

### 3.1 Ant species richness

In total, 12 ant species were collected in the four habitats over all 90 sampling plots (see Fig. 2a and Table S3). The species *Formica rufa* (one single individual worker ant found on one sampling plot in new grasslands) was excluded from the analysis as a likely stray individual. *Lasius niger* was the only species which occurred in all four habitats. Three ant species were exclusively found in old grasslands: *Lasius fuliginosus*, *Lasius alienus* agg. and *Serviformica cunicularia* .

The highest cumulated ant species richness was found in old grasslands (eight ant species, see Fig. 2b) and new grasslands (seven ant species). Species richness did not significantly differ between these two grassland habitats (see Table S4). While habitats in cereal crops (CN: three species; and CF: two species) showed significantly lower ant species richness compared to both grassland habitats ( $p < 0.05$  for all comparisons) and did not significantly differ between each other ( $p = 0.264$ ). Species loss/nestedness ( $\beta$ SNEr) accounted

for 45.83 % of the observed dissimilarity ( $\beta$ SOR) in species composition across the four habitats (see Fig. 2c and Table S5).

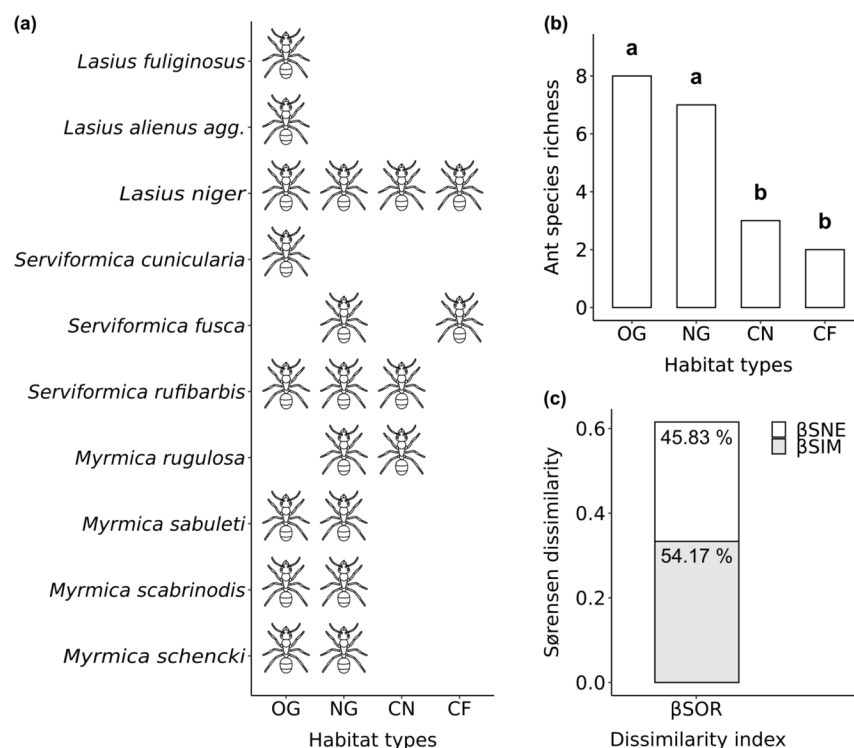


Fig. 2a-c: Ant species richness per habitat. For each of the four sampled habitat types (OG = old grassland; NG = new grassland; CN = cereal field near NG; CF = cereal field far from NG) the (a) ant species identity, the (b) ant species richness and the (c) turnover ( $\beta$ SIM) and nestedness ( $\beta$ SNE) components, and their relative contribution to the observed Sørensen dissimilarity ( $\beta$ SOR) is shown. Different letters in (b) indicate significant differences ( $p < 0.05$ ) between habitats and were calculated using a Monte-Carlo randomization test (Manly 1997).

### 3.2 Ant community composition

CAP ordination revealed that ant community composition across the transects was significantly affected by habitat type ( $p = 0.005$ , see Table S6 for PERMANOVA results). Data points referring to ant community composition of old grasslands (light green squares, see Fig. 3) were (except of one) clearly separated from other habitats, according to their position along the first ordination axis. Contrary, data points referring to ant community composition at new grasslands (dark green triangles) clustered together with data points from cereal crop habitats (purple circles and blue diamonds).

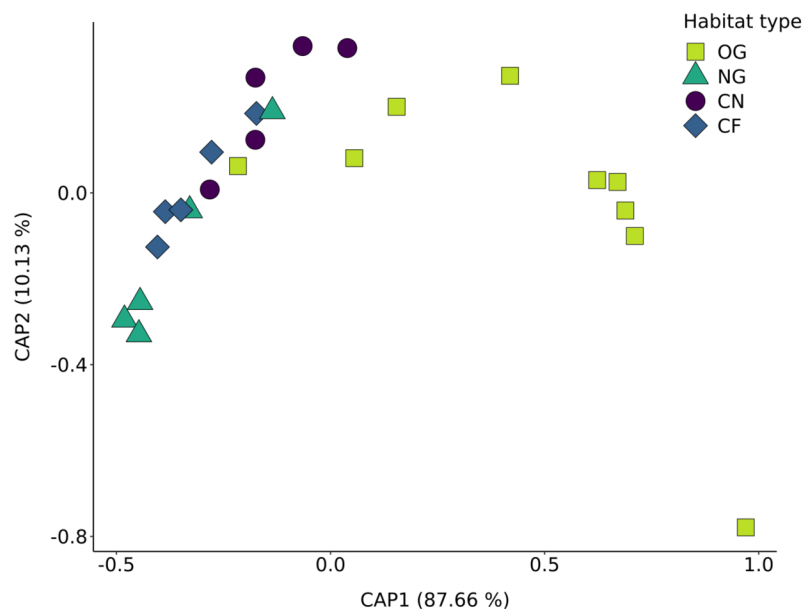


Fig. 3: Ant community composition of habitats. Ordination plot of canonical analysis of principal coordinates (CAP) showing the influence of the constraint variable habitat type (OG = old grassland; NG = new grassland; CN = cereal field near NG; CF = cereal field far from NG; differently coloured and shaped symbols respectively) on ant species composition. Each symbol indicates ant species composition of one sampled transect. Symbols have been slightly shifted to reduce overlap. Values on CAP axes refer to the percentage of explained variance (eigenvalues).

### 3.3 Principal component analysis of functional trait space

Principal component analysis showed that the functional trait space covered by ant species communities was not significantly affected by habitat type ( $p = 0.974$ , see Table S7 for PERMANOVA results). The trait spaces covered by the ant communities of cereal crops (purple dashed polygon, see Fig. 4) and new grasslands (dark green dashed polygon) showed the same distribution and were both determined by the species *Lasius niger*, *Serviformica rufibarbis* and *Myrmica rugulosa*. The species *Lasius fuliginosus* determined the only extension of the trait space occupied in old grasslands (light green dashed polygon), and the position of this species along the first PCA axis positively correlated in particular with a high affinity for nest microhabitat in wood and bark (NMW, see projected arrow in Fig. 4), social parasitism for colony foundation (SP) and large colony size (CS).



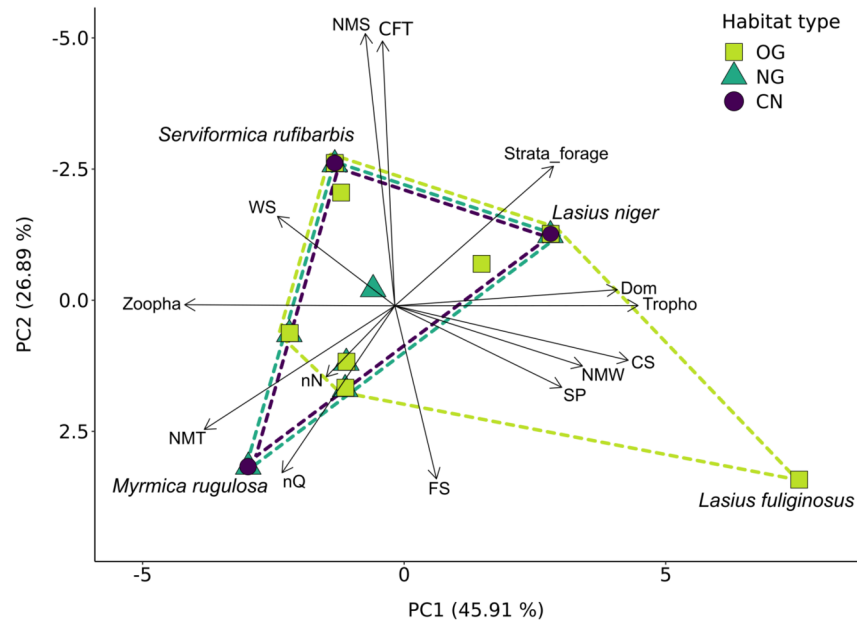


Fig. 4: Ordination plot showing the trait space covered by ant species occurring in three of the four habitat types (OG = old grassland; NG = new grassland; CN = cereal field near NG; differently coloured and shaped symbols and dashed lines respectively). CF samples (cereal field far from NG) are not shown due to low cumulative species richness. A principal component analysis (PCA) was conducted based on a species trait matrix. Each symbol indicates the position of one species occurring in the respective habitat. Names are only displayed for the most outer species (names for other species are hidden to reduce overlap). Arrows indicate the correlation of the respective traits with the species position in reduced ordination space. Used traits are as follows: vertical strata species is most likely to be found foraging (Strata\_forage), percentage of animal diet among total food intake (Zoopha), percentage of trophobiosis-based diet of total food intake (Tropho), worker body length in mm (WS), colony size log transformed (CS), behavioural dominance (Dom), number of queens per nest (nQ), number of nests per colony (nN), colony foundation type (CFT), recruitment behaviour of workers (FS), percentage of microhabitats in soil and/or under stones contributing to total nest space (NMS), percentage of microhabitats in upper root felt contributing to total nest space (NMT), percentage of microhabitats in wood and bark contributing to total nest space (NMW), colony foundation through social parasitism (SP). Values on PCA axes refer to the percentage of explained variance (eigenvalues).

### 3.4 Community weighted means of traits related to biocontrol services

The comparison of community weighted means (CWM) focussed on three species traits related to biocontrol potential of the ants and the old and new grassland habitats. Results for both cereal crop habitats are not shown, due to low cumulative species richness. CWM values of the proportion of animal based resources in ant diet (Fig. 5a) were not significantly different between old and new grasslands ( $p = 0.285$ , see Table S8) and analogous results were found for the CWM values of food recruitment strategy (Fig. 5b,  $p = 0.713$ ) and ant colony size (Fig. 5c,  $p = 0.992$ ).

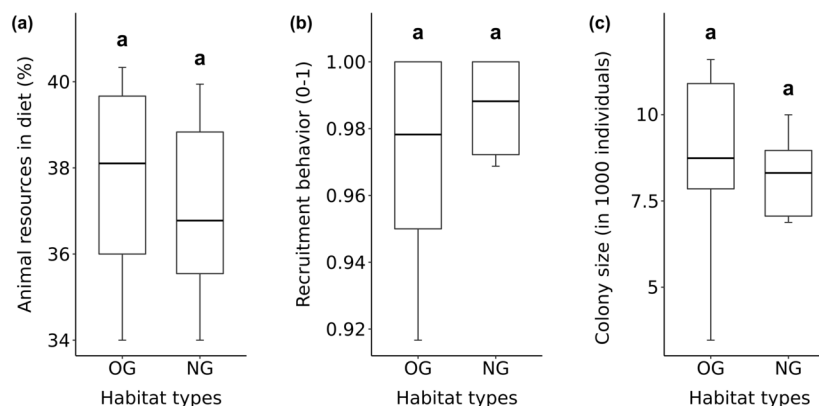


Fig. 5a-c: Biocontrol related ant species traits in old and new grasslands. For each habitat (OG = old grassland; NG = new grassland) the CWM values of the (a) proportion of animal based resources in ant diet, the (b) recruitment strategy (workers forage individually (0), workers guide a low number of nestmates to a previously discovered food source (0.5), workers can trigger mass-recruitment (1)) and the (c) colony size (in 1000 individuals) is shown. Boxes represent CIs, lines represent mean and whiskers represent ranges. Letters indicate results of Tukey's post hoc test of fixed factor levels (GLMM analysis). Different letters denote significant ( $p < 0.05$ ) differences between habitats. Results for habitats CN and CF are not shown.

### 3.5 Predation intensity on sticky cards and aboveground ant activity

Predation rate on fruit flies glued on sticky cards (see Fig. 6a) was highest for sampling plots in cereal crop far from new grasslands and lowest for new grasslands, but showed no significant differences among the four tested habitats ( $p = 0.09$  for all comparisons, see Table S9). However, predation rate was significantly lower on sampling plots with high mean vegetation cover ( $p < 0.001$ , see Table S10) and these results relate to significantly higher mean vegetation cover on sampling plots in old and new grassland habitats compared to both cereal habitats ( $p < 0.001$  for all comparisons, see Table S11).

Aboveground ant activity (number of observed workers, see Fig. 6b) was significantly highest on sampling plots in old grasslands ( $p < 0.001$ , see Table S12). Moreover, ant activity was significantly increased on sampling plots in new grasslands compared to near cereal crops ( $p = 0.016$ ) and showed a strong trend for an increase compared to cereal crops far from new grasslands ( $p = 0.0529$ ). Aboveground ant activity was not significantly different between the two cereal crop habitats ( $p = 0.905$ ). There was no significant effect of mean vegetation cover on aboveground ant activity ( $p = 0.085$ , see Table S13) and further no significant correlation between aboveground ant activity and predation intensity on sticky cards ( $R^2_{\text{m}} = 0.001$ ,  $R^2_{\text{c}} = 0.49$ ,  $p = 0.913$ ).

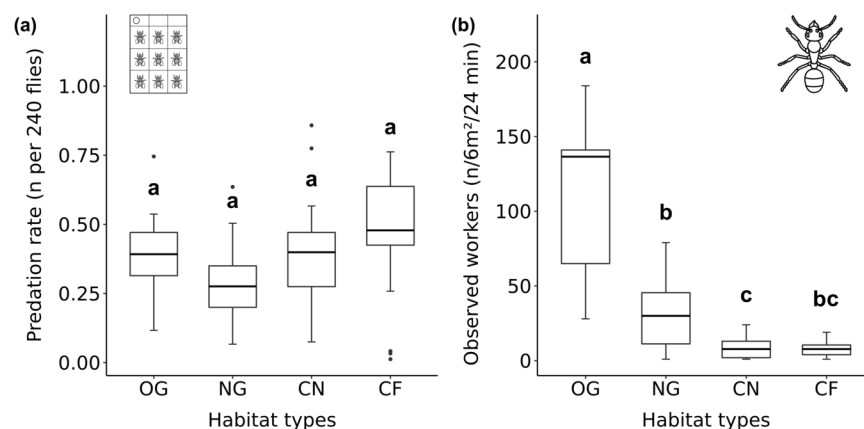


Fig. 6a and 6b. Predation rate on sticky cards and aboveground ant activity. For each habitat (OG = old grassland; NG = new grassland; CN = cereal field near NG; CF = cereal field far from NG) the (a) predation rate on sticky cards per plot summed across all 4 runs (n per 240 flies) and the (b) ant activity as the number of aboveground foraging ants per sampling plot summed across all 3 runs (observed workers per 6 m<sup>2</sup> and 24 min in total) is shown. Boxes represent CIs, lines represent mean and whiskers represent ranges. Letters indicate results of Tukey's post hoc test of fixed factor levels (GLMM analysis). Different letters denote significant ( $p < 0.05$ ) differences between habitats.

## 4 Discussion

### 4.1 Species richness and functional diversity

The results of this study demonstrate the potential of newly-established grasslands to promote ant species richness in agricultural landscapes, but only if preserved over long periods of time. Cumulated ant species richness was comparable between old and new grasslands, and significantly higher compared to surrounding cereal crops. However, even though ant species richness and abundance had already increased to high levels after three years of establishment, the new grasslands had not yet assembled ant communities of the same complexity as seen in old grassland.

Species loss or nestedness accounted for almost 50% of the variation in species composition among the habitat types, indicating that new grasslands and cereal crops habitats comprise a poor selection out of the species pool present in old grasslands. These results were supported by ordination analysis, showing that the ant community composition of new grassland transects was mostly shaped by ubiquitous agrobiont species, such as *L. niger* and a few *Myrmica* species, which are known to be resistant to anthropogenic disturbance and also inhabit cereal crops (Seifert, 2018). After three years, new grasslands were still in earlier stages of ant community succession (Dauber & Wolters, 2005), and lacked habitat specialists such as *L. fuliginosus*, *L. alienus* agg. and *S. cunicularia*. Colonies of these species require a constant supply of food resources and take several years to establish, grow and reproduce (Dauber & Wolters, 2005; Seifert, 2018).

The time lag in the colonisation of newly established grasslands by ants was also reflected by the results for the functional trait space, biocontrol experiments and biocontrol related traits in new grasslands. Principal component analysis showed that the functional richness of new grasslands was determined by three common agrobiont species (*L. niger*, *S. rufibarbis*, *M. rugulosa*), which were also present within cereal crops habitats. However, this fraction out of the local species community already provided three functional traits essential for biocontrol services, namely a predatory diet, the ability of workers to organize mass recruitment and large colony sizes. Interestingly, the extension of the functional trait space covered by ants in old grasslands was determined solely by the species *L. fuliginosus*, which is highly distinctive for many traits among the

Central European ant fauna. These ants obligately nest inside tree stems, maintain massive trail systems, attain by far the largest colony sizes of all encountered species, and, as a socially parasitic species, they depend on other ants as hosts for colony foundation.

These findings suggest that accumulating woody elements and allowing more advanced stages of vegetation succession (Dahms, Lenoir, Lindborg, Wolters, & Dauber, 2010) will help to promote functional richness and biocontrol related traits of ant assemblages in new grasslands, and thus to prevent the loss of biodiversity and associated ecosystem services in agricultural landscapes. In account of the high biocontrol potential of species such as *L. fuliginosus*, new grasslands would thereby not only extend the range of suitable foraging and nesting sites for ants (Armbrrecht, Perfecto, & Vandermeer, 2004), but further increase the contribution that ants (and other common predators such as carabids and spiders) in such habitats may provide to agroecosystem functioning in their surroundings.

#### 4.2 Predation experiments

Grasslands and cereal crops showed no difference in predation rates, but new grasslands increased ant activity compared to cereal crops. This suggests that new grasslands embedded in agricultural landscapes are able to provide a consistent amount of biological control services. Further, we could show that vegetation density influenced the outcome of sticky card experiments more than habitat type and that predation of experimentally exposed fruit flies as a proxy for pest control is generally lower on sites with higher vegetation density, such as newly established and old grasslands. These findings support the assumption that in new and old grassland transects predatory arthropods were less dependent to feed on experimentally exposed fruit flies, as the high vegetation density of these habitats likely provided a higher supply of food resources (Kruess & Tscharntke, 2002; Siemann, 1998) compared to cereal crops.

For the management of pest control it is important to consider that biocontrol services are not provided solely by ants, but by a diverse assemblage of ground-dwelling arthropod predators in the agricultural matrix (Meyer, Heuss, Feldhaar, Weisser, & Gossner, 2019). Carabids, spiders and wasps were also able to access fruit flies glued to sticky cards and their contribution, which was not assessed in this study, is most likely the reason for the lack of a correlation between predation rate and ant activity. Nevertheless, the results of the predation experiment are relevant because ants are among the most abundant predatory arthropods in certain agricultural landscapes and account for a significant part of the arthropod biomass (Wills & Landis, 2018).

#### 4.3 Synthesis and applications

Our findings show that newly established grasslands can increase ant species richness, abundance and pest control in agroecosystems, but also indicate that it takes longer than three years to maintain biodiversity level functions that are comparable to old semi-natural grasslands. To counteract the loss of important biodiversity functions, agricultural management should take into account key strategies for ecological enhancement (Bommarco, Kleijn, & Potts, 2013; Perović et al., 2018) and consider the replacement of harmful measures.

Our findings illustrate that new grasslands should be integrated into a long-term management strategy for the promotion and resilience of yield-enhancing ecosystem services provided by ants. Firstly, a turnover of newly established grasslands back into crop fields inevitably destroys initiated ant colonies, disrupts ant community succession and dramatically reduces arthropod populations that deliver key biocontrol services (Ganser, Knop, & Albrecht, 2019). Secondly, a long-term establishment of new grasslands is paramount to promote not only ubiquitous ant species in their abundance but also habitat specialists with longer colonization times (Dauber & Wolters, 2005), in account of the fact that only a broad diversity of functional insurance species can guarantee the resilience of biological control services in European agroecosystems (Tscharntke et al., 2005). Our findings suggest that newly established grasslands represent a promising measure for enhancing agricultural landscapes, but must be preserved in the longer term to allow comprehensive immigration of ant species into habitats that support agricultural biodiversity and functionality.

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