



Bumblebee footprints on bird's-foot trefoil uncover increasing flower visitation with land-use intensity



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ABSTRACT

Wild pollinators are declining in abundance, diversity and richness and this puts the ecosystem function pollination at risk. Here, we investigated how land-use intensity and the three main components of land use used on the study sites (mowing, grazing, fertilisation) affect bumblebee visitation to bird's-foot trefoil, *Lotus corniculatus*, using a novel chemistry-based approach that overcomes the limitations of classical visitation monitoring. This approach uses the hydrocarbon footprints left behind by bumblebees that accumulate within the epicuticular wax of flowers. Along with land-use intensity, we investigated whether the small-scale (patch) population density and local abundance of *L. corniculatus* affected visitation. We found that the amount of footprints, and thus visitation, of bumblebees to *L. corniculatus* increased with land-use and grazing intensity while traditionally recorded visitation rates did not show such an effect. We further found that the patch density and local abundance of *L. corniculatus* had no effect on visitation. We conclude that bumblebee visitation to *L. corniculatus* increased with land use using the novel approach but emphasise that foraging decisions by bumblebees can be affected by many different parameters unrelated to the land-use intensity of a specific site.

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1. Introduction

Pollination is an ecosystem function provided by many wild pollinator species, particularly insects, birds and bats. Without pollination by animals, many plant species that rely on pollinators for the movement of their pollen would go extinct (De Groot et al., 2002). Relative to other ecosystem functions, pollination is highly beneficial to human welfare.

However, intensification of land use has caused habitat loss, modification and fragmentation, resulting in declines in biodiversity (Pimm and Raven, 2000). Likewise, the abundance and species richness of pollinators have declined with disturbances, agricultural intensification and habitat loss. Furthermore, these changes in pollinator community may cause a reduction of pollination provided to many plant populations (Kremen et al., 2002; Natural Research Council, 2006; Ricketts et al., 2008; Winfree et al., 2009; Potts et al., 2010). Recently, Clough et al. (2014) found a decrease of insect-pollinated plants with land-use intensification. Accordingly, a parallel decline of pollinators and insect-pollinated plant species has been reported (Biesmeijer et al., 2006). Consequences of

pollinator loss are likely particularly severe for wild plant populations because ~80% depend on insect pollination and for most of these plant species pollen limitation has been observed (Burd, 1994; Ashman et al., 2004; Becker et al., 2011).

Pollen limitation can have effects on plant fecundity and ultimately population viability (Bond, 1994; Ishii and Masahiko, 2001; Lennartsson, 2002). It has been suggested that pollinators preferably visit abundant plant species so that they can maximise their rate of reward (Goulson, 2000), while pollinators are less likely to visit small populations, which as a consequence are more likely to suffer from pollen limitation (Lennartsson, 2002; Aizen and Harder, 2007; Kolb, 2008; Dauber et al., 2010). Likewise, the density (per unit area) at which plants occur might lead to differences in visitation and degree of pollen limitation. Grindeland et al. (2005) and Ye et al. (2014) found that the visitation rate of bumblebees increased with plant density.

In this study, we investigated how land-use intensity and its three main components, grazing, mowing and fertilisation, as well as small-scale patch density of *Lotus corniculatus* affected bumblebee visitation, and potentially pollination. We further investigated whether local abundance of *L. corniculatus* affected bumblebee visitation. Grassland study sites represented a land-use

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gradient and were mown, grazed or both with variable intensity, and fertilised to different degrees.

Traditionally, pollinator visitation rates have been recorded by direct observations or sometimes camera traps. The enormous amount of time needed for these methods often causes researchers to limit the number of replicate plants/sites that are investigated in their study designs as well as the choice of plant species, because for sufficient measuring accuracy a relatively high visitation rate per observed unit is required. Indeed, many studies that measured flower visitation rates in the field focused on shrubs with multiple flowers that can be observed synchronously or on other highly visited plant species (Cunningham, 2000; Kay and Schemske, 2003; Vázquez and Aizen, 2003; Greenleaf and Kremen, 2006; Winfree et al., 2008; Dauber et al., 2010; Kaiser-Bunbury et al., 2010). Here, we used a chemistry-based approach that aims at overcoming such limitations. During flower visits, bumblebees involuntarily deposit traces of cuticular hydrocarbons (CHCs). Bumblebees leave substantial amounts of CHCs wherever they walk (Wilms and Eltz, 2008) whereas non-bumblebee visitors appear to leave no or comparatively insignificant amounts of hydrocarbon footprints. Bumblebee 'footprints' accumulate within the epicuticular wax of the corolla, which consequently holds information about past bumblebee visitation (Eltz, 2006). Due to the species specificity of footprint chemical composition (Schmitt et al., 1991; Goulson et al., 2000; Saleh et al., 2007; Martin et al., 2010) deposits have also been used to assess species composition of visiting bumblebees (Witjes et al., 2011). CHCs cover insect surfaces and have the primary function to seal the cuticle against the environment, e.g. for preventing water loss (Howard and Blomquist, 2005). A secondary function is the improvement of tarsal adhesion on smooth surfaces (Lockey, 1988; Jiao et al., 2000; Drechsler and Federle, 2006). While some plant species produce alkanes in their epicuticular layer, unsaturated hydrocarbons (UHCs) are rarely found in plants but occur commonly in bumblebee footprints (Griffiths et al., 1999; Griffiths et al., 2000; Goodwin et al., 2003; Zeisler and Schreiber, 2016). Consequently, cumulative bumblebee visitation to natural flowers can be inferred from the UHCs in solvent extracts of inflorescences using Gas Chromatography/Mass Spectrometry (GC/MS) (Eltz, 2006; Witjes and Eltz, 2009).

Using this novel method, we estimated bumblebee visitation, i.e. the quantity of bumblebee footprints, on *L. corniculatus* populations in grassland sites in Germany. We chose *L. corniculatus* because it is pollinated by bumblebees (Rasmussen and Brødsgaard, 1992; Pellissier et al., 2012), is declining with land use (Weiner et al., 2014; Kämper et al., 2016) and, due to its relatively low nectar secretion rates (Stout and Goulson, 2002), has low visitation making it difficult to measure visitation rates using conventional methods. To illustrate the problems with conventional records of visitation rates, which are often dominated by many zeros and skewed by single or very few observations, we compare data collected with this new method with observations of *L. corniculatus* patches recorded on the same grassland sites.

2. Methods

Our study was conducted within the framework of the Biodiversity Exploratories (www.biodiversity-exploratories.de). For details on study regions and site selection see Fischer et al. (2010).

2.1. Data collection – classical visitor observations

To estimate visitation rates conventionally, we used previously collected data from the same region (Martina Tospaun, unpublished data). In total, 17 grassland sites were visited in the

'Schwäbische Alb' from 5-Aug-2010 to 2-Sep-2010 and 16 grassland sites in the 'National Park Hainich' from 28-Jun-2011 to 25-Aug-2011. Each grassland site was visited once, with observations conducted between 9:29 and 18:10. On 8 subplots (50 × 50 cm) per grassland site all visits to *L. corniculatus* were recorded for 15 min each. We also counted all open flowers on each subplot at the time of observation. For each subplot, we calculated the number of visits per flower per hour. We then used the average per grassland site for subsequent analyses.

2.2. Data collection – footprint-derived visitation

To assess footprint-derived UHCs of bumblebees on *Lotus corniculatus*, we visited 35 grassland sites from 04-Jun-2014 to 04-Jul-2014 and collected flowers between 15:15 and 21:15. Thirteen sites were located in the UNESCO Biosphere Reserve 'Schwäbische Alb', 15 in the National Park 'Hainich', and seven in the UNESCO Biosphere Reserve 'Schorfheide-Chorin', Germany. During each visit, we assessed the local abundance of *L. corniculatus* by counting open flowers along a 100 × 6 m transect or, if highly abundant, estimated local abundance by extrapolation from a small area.

To assess bumblebee visitation and the effect of patch density, we collected two pooled samples containing 15 *L. corniculatus* flowers on each site, respectively. For one sample, flowers were collected from *L. corniculatus* plants in low-density patches with on average 1.34 ± 0.67 *L. corniculatus* flowers per 1 m². For the second sample, flowers were collected in high-density patches with on average 28.41 ± 17.04 *L. corniculatus* flowers per 1 m². For comparison, because some plant species produce alkenes in their epicuticular wax layer (Cseke et al., 2006), we also sampled unvisited flowers. For this we covered a patch of *L. corniculatus* with mosquito mesh, removed open flowers, waited for fresh flowers to open, and took two samples of 15 flowers, both from the 'Schwäbische Alb'.

We extracted each pooled flower sample in 2 ml of n-hexane (Roth, Karlsruhe, Germany) with 10 µl of 2-undecanone (MERCK-Schuchardt, Hohenbrunn, Germany) as an internal standard for a minimum of 30 s. 30 s are sufficient to dissolve the majority of petal surface hydrocarbons along with the footprint-derived UHCs of bumblebees (T. Eltz, unpublished data). Afterwards, the inflorescences were removed and discarded. Extracts were stored at -18 °C prior to chemical analysis in Bochum, Germany, with coupled gas chromatography and mass spectrometry (GC-MS). To increase sample concentration, each extract was transferred to a 2 ml vial (Agilent, Ratingen, Germany), evaporated to dryness under a stream of laboratory air, and then filled up with 50 µl n-hexane. 1 µl of the more concentrated sample was injected splitless on a DB-5 MS column (30 m, 0.25 µm film thickness, 0.25 mm diameter). The oven of the GC (HP5890 II) was heated from 60 to 300 °C at 10° per min. A mass spectrometer (HP5972) served as detector.

Compounds were identified by comparison of mass spectra and retention times with those of reference samples (series of linear alkanes) in combination with spectral interpretation using Agilent ChemStation software (Agilent Technologies, Germany). For the quantification of hydrocarbons, we manually integrated all compounds that were potentially derived from bumblebees (based on our own data as well as Goulson et al. (2000), Martin et al. (2010) and Witjes et al. (2011)), i.e. odd-numbered alkanes, alkenes and alkadienes of a chain length of 21–31 C-atoms. The separation of the different isomers of alkenes and alkadienes, which for a given chain length have very similar spectra and retention times, was based on our own inventories of all isomers found in leg extracts of 13 bumblebee species. Alkene and alkadiene isomers of a given chain length were numbered according to their

chromatographic sequence without knowledge of their exact chemical structure, that is position or configuration of double bond (s). Isomeric peaks were assigned by using a grid of retention gaps of approximately 0.3 s length (range 0.195–0.38). For example, the peak of isomer 1 of an alkene eluted 0.01–0.31 s earlier than the start of the peak of the corresponding alkane, isomer 2 0.31–0.61 s earlier and so forth. In pooled reference samples (mixes of bumblebee leg extracts), this grid corresponded roughly to the chromatographic ‘landscape’ of alkene and alkadiene isomeric peaks.

2.3. Statistical analyses

2.3.1. Classical visitor observations

Prior to analyses the conventionally measured visitation rates were \log_e -transformed to improve normality. We then performed Spearman’s rank correlation tests to test if visitation rates were affected by land-use intensity. As a measure of land-use intensity, we used the average land-use intensity (LUI) index for 2006–2010 in the ‘Schwäbische Alb’ and 2007–2011 in the ‘Hainich’, depending on the time of data collection. For each experimental site i , the LUI_i summarises the standardised intensity of the three main components of land use, namely M_i mowing (cuts per year), F_i annual fertilisation (kg nitrogen per hectare), and G_i livestock grazing intensity (livestock units per hectare times number of days) for a given year, and F_R, M_R, G_R their respective mean within the ‘Schwäbische Alb’ or the ‘Hainich’ for that year (mean across all experimental sites) (Blüthgen et al., 2012):

$$LUI_i = \sqrt{\frac{F_i}{F_R} + \frac{M_i}{M_R} + \frac{G_i}{G_R}}$$

2.3.2. Footprint-derived visitation

For analyses of footprint-derived visitation of bumblebees, we divided the peak area of integrated ion currents of each peak by the peak area of the internal standard. We then calculated the sum of all UHCs across each sample and performed square root transformation to improve normality. We calculated four mixed models with grassland site as a random factor. While local abundance of *L. corniculatus* and patch density, i.e. the information whether a sample contained *L. corniculatus* flowers that grew in low or high density patches, were included in each model as fixed factors, each model only included one measure of land use: the average land-use intensity, mowing intensity, grazing intensity or fertilisation intensity of 2010–2014 (calculated for each region as shown above for conventionally measured visitation data). The three components of land use were square root transformed prior to analyses to improve normality, and each of the four mixed models was checked for residual normality. We included the interaction term between density and land use but excluded the term if it turned out not significant.

To reconstruct the bumblebee community based on the UHCs found on *L. corniculatus*, we used a linear model established during previous studies (Witjes et al., 2011). In our study, the linear model was based on leg extracts of 13 bumblebee species. For information on the production of leg extracts see Text A.1. In the linear model, for each *L. corniculatus* sample the amount of each UHC was solved in its own equation based on the leg extracts. To solve the system of linear equation the model calculated a least squares solution and finally, the entire model gave a maximum likelihood estimation of which bumblebee community caused the UHC profile on *L. corniculatus* per site (detailed information in Text A.2 and in Witjes et al. (2011)).

Data transformations, linear regressions and mixed models were performed with R version 3.3.0 for Macintosh OS X (R Core

Team, 2015) while the reconstruction of the pollinator community was done with a modified version of the least squares algorithm and the program ‘lsqnonneg’ in MATLAB R2015b (Mathworks Inc. version 8.6.0.267246).

3. Results

3.1. Classical visitor observations

We observed a total of 209 flower visits to *Lotus corniculatus* on 33 grassland sites. Individuals of Diptera (N = 84) were observed most frequently, followed by Hymenoptera (N = 75, *Apis mellifera* = 42, *Bombus* spp. = 26, others = 7) and Lepidoptera (N = 50). Visitation rates were generally low: no visits to *L. corniculatus* were observed on four grassland sites (12 %) and no bumblebee visits to *L. corniculatus* on 24 grassland sites (72 %). The average visitation rate of all visitors across grassland sites was 1.11 ± 1.27 individuals per hour (mean \pm SD), and the average visitation rate of bumblebees across grassland sites was 0.027 ± 0.10 individuals per hour. Based on these observed visitation rates, land-use intensity had no effect on visitation rates of either all flower visitors or bumblebees alone (Fig. 1; $\rho = -0.10$, $p = 0.597$, $\rho = 0.13$, $p = 0.472$).

3.2. Footprint-derived visitation

Using the footprint method, we found 30 different unsaturated hydrocarbons (UHCs) of the chain length of 21–31 C-atoms in solvent washes of *L. corniculatus* flowers (Table A.1). Solvent washes of unvisited flowers were free of alkenes and alkadienes. We detected footprint-derived UHCs in samples of 34 of 35 grassland sites. Summing up the area of integrated ion currents over all samples, the by far most common UHC was ‘pentacosene 4’ with 34 %, following by ‘pentacosene 6’ with 11 %, ‘tricosene 4’ with 10 % and ‘nonacosene 4’ with 9 % (Table A.1). ‘Pentacosene 4’ occurs on the cuticle of many bumblebee species and is most abundant in *B. wurflenii*, *B. lapidarius* and *B. subterraneus*, accounting for 67 %, 61 % and 35 % of UHCs found in their leg extracts, respectively (Table A.2, for information on the production of leg extracts see Text A.1). The linear model applied to reconstruct the visitor community showed that *B. lapidarius* was the most abundant bumblebee species on *L. corniculatus* (see Witjes et al. (2011), and additional information and methods in Fig. A.1, Text A.2 in Supplementary materials). Assuming that all species of bumblebees leave equal amounts of UHCs, the visits inferred from the model suggested that 46 % of visits came from *B. lapidarius*, followed by 19 % of *B. lucorum*, 13 % by *B. wurflenii*, 7 % by *B. pascuorum* and 5 % by *B. subterraneus*. The remaining 10 % of inferred visits were assigned to the remaining eight bumblebee species.

In general, land-use intensity (LUI) of grassland sites ranged from 0.48 (extensive land use) to 2.76 (intensive land use) showing that *L. corniculatus* does not occur on the most intensively used grassland sites (highest intensity level was 3.5). Bumblebee visitation, measured as summed and corrected peak area of UHCs, significantly increased with LUI (Fig. 2; mixed model with grassland site as random factor; $F = 12.78$, $df = 32$, $p = 0.001$), which means that *L. corniculatus* flowers were visited more frequently if land use was high. Investigating the composite parameters of land-use intensity, we found that mowing and fertilisation did not affect bumblebee visitation (mowing: $F = 3.30$, $df = 32$, $p = 0.079$, fertilisation: $F = 2.25$, $df = 32$, $p = 0.14$). However, we found that bumblebee visitation increased with grazing intensity ($F = 4.52$, $df = 32$, $p = 0.041$).

Although estimated bumblebee visitation in low-density patches was higher than in high-density patches in 20 out of 35

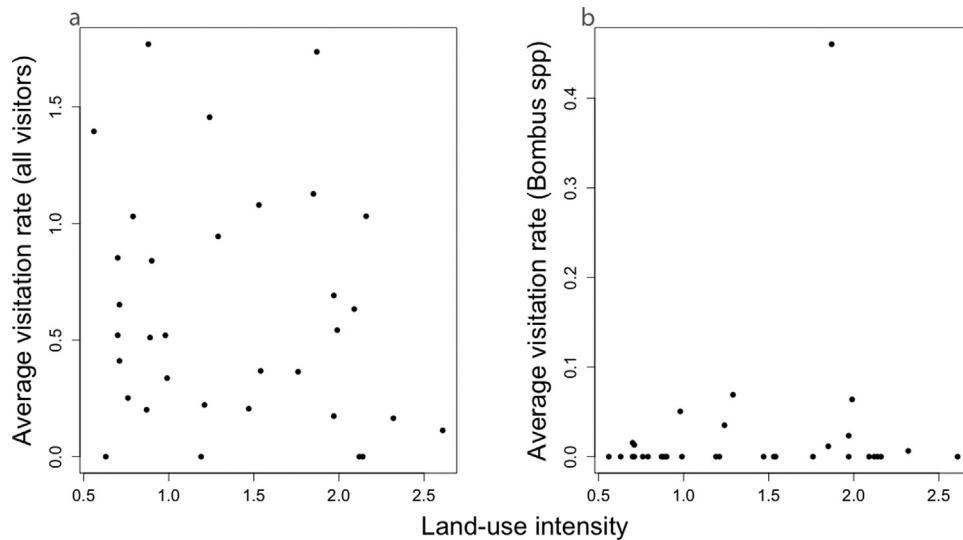


Fig. 1. Average visitation rate per hour ($\log_e + 1$) of (a) all visitors and (b) bumblebees in response to land-use intensity.

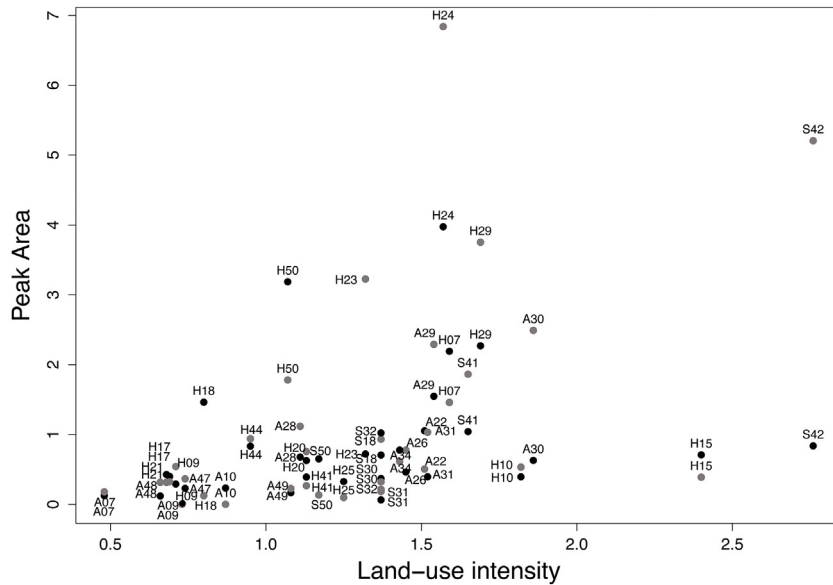


Fig. 2. Summed and ISTD-corrected peak areas of footprint-derived UHCs in solvent washes of *L. corniculatus* flowers as a function of land-use intensity of the grassland sites. Black circles indicate that flowers were collected from high-density patches, grey circles that they were collected from low-density patches. The labels on each circle indicate the name of the grassland site, with 'A' for the Biodiversity Exploratory 'Schwäbische Alb', 'H' for Hainich and 'S' for 'Schorfheide-Chorin'.

cases, patch density of *L. corniculatus* did not consistently affect bumblebee visitation (Fig. 2; $F = 0.60$, $df = 34$, $p = 0.442$). The local abundance of *L. corniculatus* had no effect on bumblebee visitation ($F = 0.40$, $df = 32$, $p = 0.530$).

4. Discussion

Using bumblebee-derived hydrocarbons on flowers (footprints) as an indicator for bumblebee visitation, we found that the visitation of bumblebees to *Lotus corniculatus* unexpectedly increased with land-use intensity, while traditionally recorded visitation rates failed to show such an effect. In more detail, we found that visitation of bumblebees to *L. corniculatus* increased with grazing intensity whereas mowing and fertilisation intensity had no effect. It seems likely that this discrepancy is due to the low accuracy of our observational visitation data on a plant species that

receives low visitation rates: a total of 26 bumblebee visits across all 35 sites are far too few to accurately estimate visitation rates by bumblebees that are known pollinators of *L. corniculatus* (Rasmussen and Brødsgaard, 1992; Pellissier et al., 2012). The limited observation time per site represents a common problem in multi-replicate studies of flower visitation. In contrast, unsaturated hydrocarbons (UHCs), identical to the ones found on bumblebee cuticle, were present in measurable quantities in samples of all but one grassland site. The footprint method requires only a single visit of the researcher per site to collect samples that integrate bumblebee visitation over the past several days and thus, for bumblebees, overcomes the limitations of traditionally recorded visitation rates. The average time the sampled flowers had been open to visits at the time of sampling, is unknown, but it is most likely at the scale of a few days. The blooming of individual flowers of *L. corniculatus* was found to last about 7 days in UK grasslands

(Ollerton and Lack, 1998). Arguably, the actual longevity of the sampled flowers will also depend upon temperature, humidity, nutrient availability and other factors affecting plant performance, and perhaps also pollination (Primack, 1985; Yasaka et al., 1998; Marques and Draper, 2012; Arroyo et al., 2013). However, it seems likely that amounts of UHCs provided a more accurate estimation of site-specific bumblebee visitation rates than did direct observations for plants with low number of individual visitors per hour. Previous studies showed that hydrocarbons deposited by bumblebees remain on the corollas of visited plants for up to 48 h without substantial loss (Witjes and Eltz, 2009), and possibly much longer, suggesting that the footprint method can cover the range of expected flower exposure times in *L. corniculatus*.

However, an alternative explanation for the discrepancy between the traditionally recorded visitation rates and the footprint-derived estimation of flower visitation could be that the data were collected in different months and years. Foraging preferences of bumblebees vary over time and season likely due to differences in the flower community (Requier et al., 2015) and the current nutritional needs of the colony (Kitaoka and Nieh, 2009). Consequently, sampling/observing at other times during the *L. corniculatus* flowering period might have yielded different results, potentially decreasing the inconsistency of the two types of visitation measures.

Unfortunately, footprint-derived estimation of flower visitation cannot easily be extrapolated to non-bumblebee visitors that appear to leave no or comparatively insignificant amounts of hydrocarbon footprints. Bumblebees leave substantial amounts of UHC wherever they walk (Wilms and Eltz, 2008), and all long-chain UHCs that we found on *L. corniculatus* could be assigned to bumblebee origin. Neither in the present, nor in previous footprint studies (Eltz, 2006; Witjes and Eltz, 2009; Witjes et al., 2011), were we aware of hydrocarbons that clearly indicated the presence of other groups of insects, e.g. flies or beetles. However, all these studies focussed on plants typically visited and pollinated by bumblebees, or at least by bees. Honeybees (*Apis mellifera*) cuticular hydrocarbons broadly overlap with those of bumblebees (Châline et al., 2005; Witjes et al., 2011), and, indeed, some of the UHCs on *L. corniculatus* may have been derived from honeybees. However, honeybees do carry substantially lower quantities of hydrocarbons on their cuticles in comparison to bumblebees (W. Kämper & T. Eltz, unpublished data). In footprint experiments they also deposited much smaller quantities of UHCs (one to two orders of magnitude less) than the buffed-tailed bumblebee, *B. terrestris*, on natural flowers (*Borago officinalis*) and on glass slides fixed to the nest entrance (T. Eltz, unpublished data). We therefore believe that the UHCs washed from *L. corniculatus* flowers in the present study are almost entirely derived from bumblebees, and that the contribution of honeybees or other insects was negligible.

The positive land use effect on bumblebee visitation to *L. corniculatus* was in contrast to our prediction. Several studies have shown that the abundance and richness of pollinators declined with disturbances and habitat loss, and as a consequence it was suggested that these declines entail a reduction of pollination as an ecosystem function (Kremen et al., 2002; Natural Research Council, 2006; Ricketts et al., 2008; Winfree et al., 2009; Potts et al., 2010). Some studies suggested that the decline of pollinators might have particularly severe consequences for wild plant populations, because in many wild plants reproduction is limited by pollination (Burd, 1994; Ashman et al., 2004; Becker et al., 2011). From former studies on the same grassland sites, we knew that *L. corniculatus* is negatively affected by land-use intensification (Weiner et al., 2014; Kämper et al., 2016), and in general a decline in density of insect-

pollinated grassland plants was found with land-use intensification (Clough et al., 2014). Likewise, *B. lapidarius* who is the most common visitor of *L. corniculatus* on the grassland sites used for this study, has been shown to occur at lower abundances on more intensively used grassland sites (Weiner et al., 2014; Kämper et al., 2016). Nonetheless, our study showed that the visitation to the wild plant *L. corniculatus* did not suffer from increased land-use intensity but instead increased. However, we doubt that this is a result of higher bumblebee abundances. Instead, we believe that the increase in bumblebee visitation to *L. corniculatus* with increasing land use might have been caused by a lack of alternative forage or increased availability of *L. corniculatus* in the entire flight radius of bumblebees making it a valuable food source. The reported declines of *L. corniculatus* abundance with land-use intensity are thus likely driven by other factors unrelated to pollination, most likely resource limitation. Stephenson (1984) found that resources rather than pollination limit the reproductive output of *L. corniculatus*. Alternatively, vegetative competition might occur, as shown for *Limnanthes* species that experience strong fertility barriers when dispersing into sites occupied by congeners (Runquist and Stanton, 2013).

In the attempt to elucidate the mechanism causing the increase in visitation with land use, we found that local abundance and patch density of *L. corniculatus* did not affect footprint-derived bumblebee visitation. This was partly contrary to our expectations because from a bee's perspective it is beneficial to forage on plants that are abundant on relevant scales in order to maximise foraging efficiency (Goulson, 2000). For limited bumblebee population densities in a site, however, these visitors may not adequately respond to higher flower abundances, constraining overall visitation rates. Note that all grassland sites included in this study had non-marginal populations of *L. corniculatus*, without which we could not have implemented our paired patch density design. If sites with isolated single individuals had been included to represent a broader gradient of densities, an effect would seem more likely. On the smallest spatial scale, we expected visitation to increase with patch density, i.e. the density of conspecific plants/flowers in the immediate vicinity, at which *L. corniculatus* occurred because it has been shown that the visitation rate of bumblebees was higher on dense patches of *Digitalis purpurea* and *L. corniculatus* than on sparse ones (Grindeland et al., 2005; Ye et al., 2014). However, in our study we did not find such an effect, possibly due to smaller differences between low and high-density patches compared to those reported by Ye et al. (2014).

Finally, we conclude that footprint-derived bumblebee visitation increased with land-use intensity of a site while traditionally recorded observational visitation rates did not show such an effect. However, we emphasise that the foraging decisions by bumblebees can be affected by many different parameters unrelated to the land-use intensity of a specific site.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.agee.2017.02.013>.

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