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RESEARCH ARTICLE

How landscape, pollen intake and pollen quality affect colony growth in *Bombus terrestris*

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Abstract
Context Abundance and diversity of bumblebees have been declining over the past decades. To successfully conserve bumblebee populations, we need to understand how landscape characteristics affect the quantity and quality of floral resources collected by colonies and subsequently colony performance.
Objectives We therefore investigated how amount and composition of pollen collected by buff-tailed bumblebee *Bombus terrestris* colonies was affected by the surrounding landscape (i.e. the proportion of forest, urban, semi-natural habitats) and how they were related to colony growth.
Methods Thirty *B. terrestris* colonies were placed at grassland sites differing in surrounding landscape. Colonies were established in spring when availability of flowering plants was highest, and their weight gain was monitored for 1 month. We additionally recorded the quantity and compared plant taxonomic composition and nutritional quality (i.e. amino acid composition) of pollen stored.
Results Bumblebee colonies varied little in the pollen spectra collected despite differences in surrounding landscape composition. They collected on average 80 % of pollen from woody plants, with 34 % belonging to the genus *Acer*. Early colony growth positively correlated with total amount of woody pollen and protein collected and decreased with increasing proportions of semi-natural habitats and total amino acid concentrations.
Conclusions Our results suggest that woody plant species represent highly important pollen sources for the generalist forager *B. terrestris* early in the season. We further show that colony growth of *B. terrestris* is predominantly affected by the quantity, not quality, of forage, indicating that several abundant plant species flowering throughout the bumblebees' foraging season may cover the colonies' nutritional needs.

Keywords Amino acids · Biodiversity exploratories · Floral resources · Foraging · Generalist pollinators · Landscape · Nutrition · Protein

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Introduction

In temperate regions, bumblebees are amongst the most important pollinators of crops and wildflowers (Goulson 2010). Over the last decades, the abundance and diversity of these wild pollinators have been declining, likely due to reductions in and alterations of their preferred habitat (Potts et al. 2010; Goulson et al. 2015) and associated changes in flower composition and diversity (Biesmeijer et al. 2006; Clough et al. 2014).

So far, almost all studies that investigated the impact of landscape characteristics on bumblebees either measured individual bee abundance, species diversity (Steffan-Dewenter et al. 2002; Westphal et al. 2003; Kleijn and van Langevelde 2006; Carvell et al. 2011; Klein et al. 2012), or nest density (Knight et al. 2009; Goulson et al. 2010). These studies found a positive correlation between the abundance of several bumblebee species and flowering plant diversity as well as with the presence of particular plant families (Williams 1986; Mänd et al. 2002; Hines and Hendrix 2005; Goulson 2010; Hülsmann et al. 2015), pointing to a close relationship between flowering plant diversity and the (nutritional) quality of floral resources collected by bumblebees. However, while individual bumblebee abundance and diversity relate to the attractiveness of a patch or field for foragers at the moment of observation, they do not allow for assessing the effect of the surrounding landscape and pollen parameters on colony growth and development. Studies that investigated how bumblebee colony growth is affected by landscape found that environments with abundant resources (Westphal et al. 2006; Crone and Williams 2016) and fields of mass flowering oilseed rape (Westphal et al. 2009) enhanced colony growth and that suburban habitat increased colony weight gain and final nest size compared to conventionally farmed and improved habitats (Goulson et al. 2002).

However, as none of the above studies has analysed resource use of colonies, we still do not know how landscape characteristics affect the floral composition, quantity and quality of resources collected by bumblebees. Adult bumblebees as well as larvae rely completely on floral resources, i.e. pollen and nectar, for nutrition (Michener 2007). While nectar is the main energy source particularly for adult workers, pollen provides not only protein but also lipids, vitamins and minerals essential for brood production (Haydak 1970; Brodschneider and Crailsheim 2010). The total protein content as well as the composition of different amino acids vary between different plant species (Roulston et al. 2000; Weiner et al. 2010), but the amount and proportions of amino acids considered essential for honeybees (De Groot 1953) and bumblebees (Génissel et al. 2002) is comparatively similar across plant taxa (Roulston and Cane 2000; Weiner et al. 2010). The survival and immune function of individual workers as well as the performance of entire colonies increases with the protein content of dietary pollen in both honeybees and bumblebees (Regali and Rasmont 1995; Génissel et al. 2002; Tasei and Aupinel 2008; Di Pasquale et al. 2013; Brunner et al. 2014; Vanderplanck et al. 2014). Moreover, despite differences in their forage spectra, different bumblebee species collect a pollen diet of comparatively high protein and essential amino acid content (Leonhardt and Blüthgen 2012). Besides other constituents (Di Pasquale et al. 2013), pollen protein content, amino acid composition and pollen quantity thus appear to play an essential role for the performance and fitness of generalist bees, such as bumblebees, and both pollen quality and quantity may be directly linked to landscape related differences in flowering plant composition.

In the present study, we investigated how landscape interacts with the taxonomic composition, quantity and quality of pollen collected by the buff-tailed bumblebee *Bombus terrestris* and how these parameters affect early colony growth, which is a prerequisite for, but does not equal, bumblebee reproduction (i.e. production of gynes and males) and population growth (i.e. increased number of colonies). We placed 30 commercially reared buff-tailed bumblebee colonies (*Bombus terrestris*) at 30 grassland sites differing in the composition of the surrounding landscape, monitored their growth over 1 month and analyzed the quantity and quality of pollen collected.

Based on previous findings showing that the proportion of forest, urban and semi-natural habitat were positively correlated with bumblebee colony growth, we hypothesized that increasing proportions of these habitats enhance colony growth by providing more pollen of higher nutritional quality and diversity. We further predicted that fast and slow growing colonies differ in the floral and nutritional pollen spectra collected.

Methods

Study sites and colonies

All grassland sites are part of the Biodiversity Exploratory project (www.biodiversity-exploratories.de). For details on study regions and site selection see Fischer et al. (2010). The project provides information on land cover types surrounding the experimental grasslands, which were mapped based on high-resolution aerial photographs and an extensive field mapping campaign in 2009 (see Steckel et al. 2014 for details). Land cover, i.e. the percentage cover of bumblebee-attractive habitats like forest, urban and semi-natural habitats (Goulson et al. 2002, 2010), was calculated with FRAGSTATS 3.3 (McGarigal et al. 2002) for concentric circles of 500 m radius around the experimental grassland sites. Proportion of forest was the total forest cover and contained all types of forests except commercially managed forestries (mean area percentage ± SD: 36 ± 21 %). Proportion of urban contained all types of settlements excluding roads (3 ± 4 %). Semi-natural habitats consisted of habitats of extensive land use, such as extensively managed meadows, marshland, shrubland, hedges (>5 m width), calcareous grasslands and orchards (11 ± 15 %, for details see Supplementary Information). We used data from only 1 year (2009), because forests as well as urban and semi-natural habitats typically persist over long periods with only minor alterations between years. To assess whether our colonies had access to and, if so, foraged on pollen from mass flowering crops (Westphal et al. 2003, 2009), we additionally recorded whether fields with oilseed-rape (*Brassica napus*) were present within the 500 m radius around colonies and whether they were in flower. Six out of our 30 colonies had access to flowering oilseed-rape during the experimental period.

In 2013, 30 colonies of the buff-tailed bumblebee (*Bombus terrestris*) were purchased from STB Control (Aarbergen, Germany) and transferred to 30 randomly chosen experimental grassland sites in the Schwäbische Alb, Germany (Fig. 1). Experimental sites were approximately 150 × 150 m and on average 7360 m apart (range: 204–20,253 m). The 500 m radius of six plot-pairs overlapped. All bumblebee colonies were exactly 4 weeks old and weighed 566 ± 33 g when transferred to the sites. They were placed in the center of each experimental grassland site in spring on May 7th and 8th 2013, when availability of flowering plants is highest, and re-collected on June 8th and 10th 2013. To protect the colonies from moisture (relative humidity per month: 91 ± 5 %; air temperature per month: 9 ± 0.2 °C, monthly average across plots for May 2013, provided by the Biodiversity Exploratory project) we placed them on brick stones and covered the boxes with sheets of polystyrene foam, weighed down with brick stones. At the beginning and the end of the experimental period, all nest boxes were weighed. At the end of the experiment, all colonies were placed in a freezer at −18 °C to kill bumblebees and preserve pollen stores for subsequent analyses.

Sample preparation

Colonies were defrosted at room temperature. Weight of workers, drones, gynes and brood was recorded, but not analyzed, because these variables were all correlated with weight gain. We separated pollen pots from all other nest structures. To obtain all pollen and to determine the weight of pollen stored per colony, we added 500 mL of ultrapure water to all pollen pots and wax residues obtained from one colony and boiled this mixture for up to 1.5 h until all wax was dissolved. The mixture was then sieved to remove larger particles that did not dissolve, e.g. cotton wool. After 24 h, the pollen had sedimented to the bottom. The supernatant was discarded. Remaining water was removed by centrifugation (8 min at 1500 rpm) and by spreading pollen in a glass container for drying (72 h). The dry pollen was weighed and approximately 10 mg were set aside for the amino acid analysis.

Plant source identification by pollen grain analysis

We used approximately 7 mm^3 of pollen from each colony for pollen grain analysis and followed standard palynological protocols (Erdtman 1954; Hesse and Waha 1989). Briefly, pollen was mixed with 1 mL 10 % KOH solution in Eppendorf tubes (2 mL) and boiled at 90 °C (15 min) in a thermoblock (Ts1, Thermoshaker, Biometra). The suspension was centrifuged (3 min at 1300 rpm) and the supernatant discarded. Subsequently, we added 1 mL acetic acid to the pollen, centrifuged and again discarded the supernatant. Sulphuric acid (J.T. Baker, Deventer, NL) and acetic anhydride (Roth, Karlsruhe, Germany) (1:9) were then added to the pollen mixture, boiled at

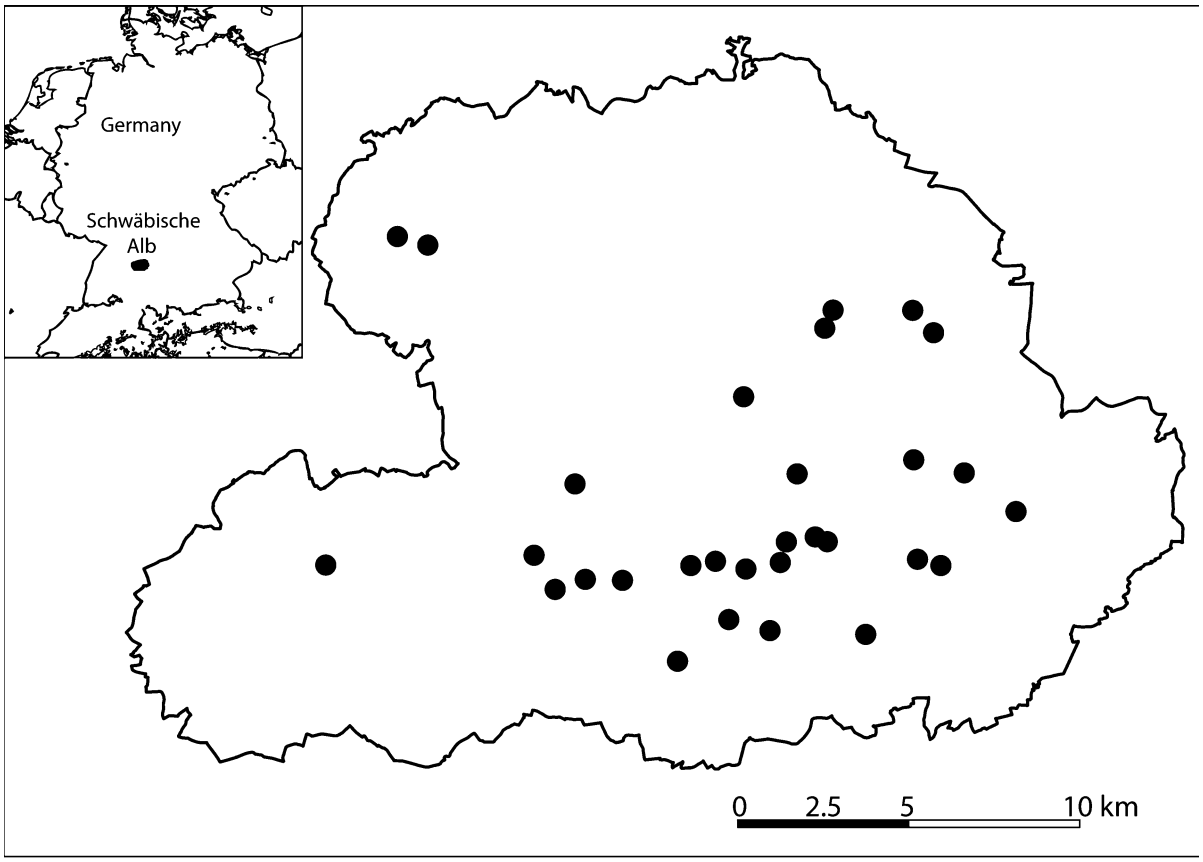

Fig. 1 Map of Germany and the study area 'Schwäbische Alb', which is part of the Biodiversity Exploratory project. For details on the study region see Fischer et al. (2010)

90 °C (3 min) and again mixed with acetic acid (J.T.Baker, Deventer, NL) before washing each sample twice with 1 mL distilled water. The solution was stirred and centrifuged (3 min at 13,000 rpm) in between.

Permanent slides were prepared by mixing the pollen sediment with Kaiser's glycerol gelatine (Merck, Darmstadt, Germany). The mixture was stirred and a few drops were pipetted on a microscope slide, covered with glass slips and sealed with clear varnish to avoid contamination. Pollen spectra were characterized under a microscope (Zeiss, Jena, Germany) at 200–400× magnification. In a first step, the slides were screened for different pollen morphotypes, and each new type was measured, photographed with a stereo microscope (Olympus BX40 with coupled with Altra 20) and entered into our pollen database. Pollen types were identified to variable taxonomic levels following Beug (2004) and the pollen guide published by the Lower Saxony State Office for Consumer Protection and Food Safety (von der Ohe and von der Ohe 2007). Additionally, we compared samples to a reference collection of pollen obtained directly from flowering plants at the Schwäbische Alb during the same field season (54 species of grassland angiosperms visited by bumblebees). The abundance of each pollen type on a slide was assessed based on its volume, similar to the approach used by Biesmeijer et al. (1992) and Eltz et al. (2001). This approach corrects for pollen size differences between different species. The specific volume of each pollen type was calculated as suggested by Neumayer and Paulus (1999), i.e. for an elongated pollen grain by adding the volume of a cylinder and two hemispheres. Grains were then counted in quadrants along transects across the center of each slide, and the cumulative volume for each pollen type as well as the total volume of all pollen types were calculated. For a given slide we

stopped counting at 6,000,000–8,000,000 μm^3, which represented on average 311 ± 24 pollen grains. Absolute volumes of each pollen type were divided by total volume of all pollen types counted per slide, yielding relative volume proportions for each pollen type and slide.

Amino acid analysis of pollen

Amino acids of pollen collected from nests were analyzed by ion exchange chromatography (IEC: Biochrom 20 plus) as described in Leonhardt and Blüthgen (2012). Approximately 10 mg pollen (9.8 ± 0.6) of each colony were dried and then mixed with 200 µL of 6 N HCl, boiled for 4 h at 100 °C, cooled down to room temperature and centrifuged (10 min at 14,800 rpm). The supernatant was transferred into a fresh tube and water was evaporated at 100 °C before the sample was re-dissolved in 200 µL of deionized water and evaporated once more. Afterwards the sample was again re-dissolved in 200 µL of deionized water and centrifuged (10 min at 14,800 rpm). Then, 100 µL of the supernatant was mixed with 20 µL of 12.5 % sulphosalicylic acid, extracted in the refrigerator (30 min), mixed and centrifuged again (10 min at 14,800 rpm). Finally, 100 µL of the supernatant was mixed with 100 µL sample rarefaction buffer in a fresh microcentrifuge tube, filtered and centrifuged (5 min at 10,000 G = 11,641 rpm) before the sample was transferred into a fresh microcentrifuge tube for further rarefaction with buffer (1:10) and analyzed by IEC.

Statistical analyses

To relate colony growth to the diversity of flowering plant species visited for pollen collection, we calculated effective pollen morphotype diversity ($e^{H'Pollen}$) for each colony by exponentiating Shannon diversity. In doing so, we obtained the effective number of interacting partners instead of using Shannon entropy (see Jost 2006). To investigate whether fast and slow growing colonies differed in the floral spectra collected, we additionally analyzed the colony-pollen network, i.e. a matrix representing the interactions between different colonies and pollen morphotypes based on the proportion of each pollen morphotype recorded standardized for each colony. We calculated the network specialization indices H_2' and d' (Blüthgen et al. 2006). H_2' characterizes the degree of floral specialization across colonies with regard to pollen sources. It ranges from 0 (pollen samples from all colonies have the same relative composition of pollen types) to 1 (each colony collects pollen with a unique pollen spectrum). The d' index is obtained for each colony and indicates how strongly a colony deviates from the pollen choices of other colonies. It also ranges from 0 (complete overlap) to 1 (exclusive pollen types). Because pollen from woody plants appears to be particularly important for honeybees (Odoux et al. 2012; Requier 2013) and wild bees (Bailey et al. 2012) in spring, we further estimated the total amount of woody pollen from relative volume proportions collected by each colony.

We used piecewise SEM to investigate direct and indirect relationships between explanatory variables and colony weight gain based on a priori knowledge of theorized interactions with a sample size of 30. Our piecewise SEM was fitted using linear models to investigate how colony growth over the experimental period (measured as weight gain in g) was affected by (i) proportion of forest/urban and (ii) proportion of semi-natural habitats, via (iii) effective pollen morphotype diversity ($e^{H'Pollen}$), (iv) total amount of woody pollen per colony and (v) total amino acid concentration (µg/mg dry weight) of stored pollen per colony. Furthermore, interaction pathways between (iii) and (iv) and total amino acid concentration were included.

Because piecewise SEM loses robustness for covarying variables (Lefcheck 2015), we only included variables that did not significantly correlate (Spearman's rank correlation). Due to a strong negative correlation between areas of forest and urban habitat ($r = -0.72$, $p < 0.001$), we composed separate models for each variable. Proportions of forest, urban and semi-natural habitats were square root-transformed to improve residual normality.

To determine whether single pollen morphotypes or single amino acids played a potential role for colony growth, we performed redundancy analyses (RDA) with colony growth included as the first (fixed) axis. For both pollen morphotypes and amino acids we first standardized our data by calculating proportions of occurrence within each colony. Proportions were obtained by dividing the volume of single pollen

morphotypes or concentrations of each amino acid in a colony sample by the total pollen morphotype volume or amino acid concentration summed for all pollen morphotypes or amino acid concentrations, respectively, in this sample. To test whether the concentration of all essential amino acids (De Groot 1953) in pollen and/or the total amount of amino acids (i.e. total protein) in a colony correlated with colony growth, we performed additional Spearman's rank correlation tests. The total amounts of amino acids in a colony were obtained by multiplying the total amino acid concentration with the weight of pollen stored.

All statistical analyses were done with R version 3.1.1 for Macintosh OS X (R Core Team 2015). For the network graph we used the R package 'bipartite' (Dormann et al. 2009), for the piecewise structural equation model 'piecewiseSEM' (Lefcheck 2015) and 'MuMIn' (Bartoń 2016), and for the RDA 'vegan' (Oksanen et al. 2015).

Results

Colony development and pollen storage

Even though the weather in May 2013 was extremely harsh with a lot of rain and local flooding, all *Bombus terrestris* colonies developed well and on average doubled their net weight (average ± SD increase of 589.46 ± 255.77 g) over the 31 days of our experimental period. Brood accounted for on average 25 % of the final weight (285.57 ± 131.56 g), followed by 3 % weight of workers (40.11 ± 17.59 g), 2 % weight of pollen stores (26.63 ± 14.66 g), 2 % weight of gynes (19.41 ± 43.27 g), and 0.5 % weight of drones (5.55 ± 8.15 g).

At the end of the experimental period, we identified 23 different plant families and 35 pollen morphotypes in the colonies' pollen stores (Table 1; Fig. 2). Two morphotypes could not be assigned to any plant taxon. Among the 23 plant families, Rosaceae and Sapindaceae were the most prominent, representing 27 and 36 % of the total pollen volume, respectively. Woody plants were in general far more common in pollen stores than herbaceous plants, accounting for on average 80 ± 16 % of stored pollen volumes in each colony (Table 1; Fig. 2). Note that *Brassica napus*, which was flowering close to at least 6 of our 30 colonies, was not among the stored pollen types.

The diversity of plant families and pollen morphotypes ($e^{H'Pollen}$) in the pollen stores was generally low with an average effective number of 5 ± 1 families and 7 ± 1 morphotypes. Different colonies were similar in their pollen choices with the degree of quantitative partitioning being relatively low ($H_2' = 0.12$). Specialization of individual colonies was also low, with no colony deviating much from random sampling (mean $d' = 0.07 \pm 0.04$). None of the colonies used exclusive pollen morphotypes, which were not also used by other colonies, except for Salix, and two unknown pollen morphotypes, which were only collected by one colony each. However, these morphotypes were only collected in minute quantities (Table 1). The by far most abundant pollen morphotype was the woody plant morphotype *Acer,* which was present in all 30 colonies, representing on average 34 ± 1.08 % of a colony's pollen volume.

The average amino acid concentration of the pollen stored was 104.60 ± 22.64 μg/mg (dry weight). The most abundant amino acids in the pollen stores were alanine, aspartic acid, glutamic acid, glycine and the essential amino acid leucine, each accounting for 9–12 % of all amino acids with a concentration between 7 and 13 μg/mg pollen dry weight (Table 2).

Variables affecting colony growth

The composed piecewise SEM represented our data sufficiently well (model including forest habitat: $p = 0.54$, AICc = 235.03, model including urban habitat: $p = 0.49$, AICc = 235.48). In both models, the proportion of semi-natural landscapes at a 500 m radius, the amount of woody pollen collected, and pollen quality explained most of the observed variance (Fig. 3). Generally, the proportion of semi-natural habitat as well the quality of the stored pollen, measured as total amino acid concentration, negatively correlated with *Bombus terrestris* colony growth. However, total amount of woody pollen positively correlated with colony growth (Fig. 3) while no single pollen morphotypes contributed specifically to colony growth (see RDA in Fig. 4a). No correlation was found between colony growth and either pollen morphotype diversity, or the proportion of forest (Fig. 3a), or urban habitat (Fig. 3b).

We further found no correlations between proportion of semi-natural habitat or forest/urban habitat and

Table 1 Pollen volume and standard deviation (SD) (%) as well as the plant family and type of all pollen morphotypes found in pollen stores of 30 *Bombus terrestris* colonies experimentally placed at 30 grassland sites

Plant morphotype	Plant family	Pollen volume ± SD (%)	*N*
Woody plants		SUM: 79.33	
Acacia	Fabaceae	0.17	4
Acer	Sapindaceae	34.45 ± 1.08	30
Aesculus	Sapindaceae	1.30 ± 3.68	26
Castanea	Fagaceae	0.01 ± 12.47	7
Ericaceae	Ericaceae	0.1	4
Lonicera	Caprifoliaceae	14.93 ± 2.18	30
Pinus	Pinaceae	$0.63 \pm 3 \times 10^{-7}$	6
Rosaceae type 1	Rosaceae	12.74 ± 2.08	29
Rosaceae type 2	Rosaceae	14.58 ± 1.11	30
Salix	Saliaceae	0.01	1
Tilia	Malvaceae	0.41 ± 19.41	8
Herbaceous plants		SUM: 20.65	
Allium	Amaryllidaceae	0.03 ± 13.68	4
Brassicaceae	Brassicaceae	2.48 ± 4.63	29
Campanula	Campanulaceae	0.10 ± 6.45	6
Centaurea	Asteraceae	0.23 ± 31.59	4
Cistus	Cistaceae	3.05 ± 3.15	22
Crepis	Asteraceae	0.28 ± 5.21	14
Echium	Boraginaceae	0.004 ± 10	4
Geum	Rosaceae	0.30 ± 8.03	10
Heracleum	Apiaceae	0.11 ± 10.40	7
Lamium	Lamiaceae	1.82 ± 1.99	29
Medicago	Fabaceae	0.24 ± 16.70	6
Plantago	Plantaginaceae	0.12 ± 9.95	5
Ranunculus	Ranunculaceae	2.68 ± 2.30	29
Resede	Resedaceae	0.36 ± 4.07	21
Rhinanthus	Orobanchaceae	5.35 ± 2.52	28
Salvia	Lamiaceae	0.02 ± 23.57	2
Senecio	Asteraceae	0.14 ± 6.43	11
Silene	Caryophyllaceae	0.08 ± 35.36	2
Symphytum	Boraginaceae	0.54 ± 44.09	3
Trifolium	Fabaceae	0.69 ± 3.48	18
Vicia	Fabaceae	1.78 ± 3.33	24
Viola	Violaceae	0.23 ± 16.67	4
Unknown type 1	Unknown	0.01	1
Unknown type 2	Unknown	0.01	1

N gives the number of colonies, which had foraged on each pollen morphotype. *Acacia*, Ericaceae, *Salix* and the two unknown pollen morphotypes do not have SDs, because we found only one pollen grain per colony sample for each of those morphotypes

the amount of woody pollen or pollen quality collected. Moreover, we found no correlations between pollen morphotypes diversity, the amount of woody pollen and pollen quality. However, in the model including forest habitat, pollen morphotypes diversity ($e^{H'Pollen}$) negatively correlated with the proportion of semi-natural habitat (Fig. 3a), whereas this correlation was only marginally significant in the model including urban habitat ($p = 0.08$; Fig. 3b).

The concentration of all essential amino acids was negatively correlated with colony growth ($r = -0.52$, $p = 0.003$). However, colony growth increased significantly with total amounts of amino acids stored in form of pollen, calculated by multiplying amino acid

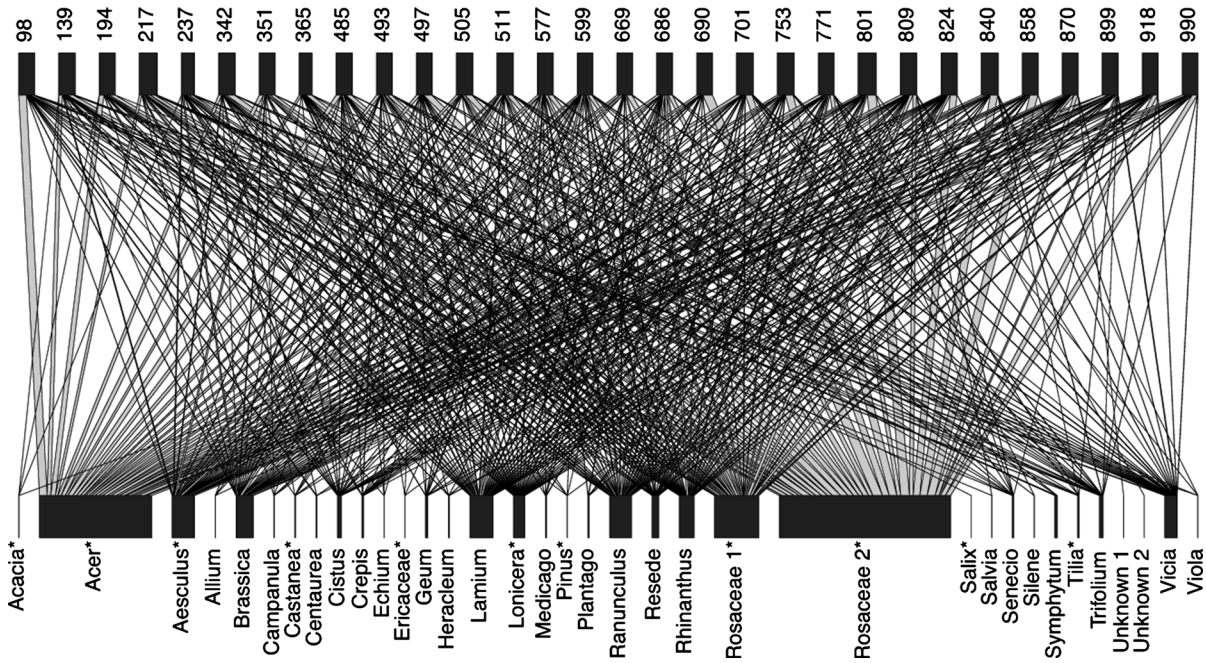

Fig. 2 Pollen collection network of 30 *Bombus terrestris* colonies. Block sizes and values on top show colonies with their weight gain at the end of the exposure [in g]. Block sizes down below give the averaged proportion for each pollen morphotype as found across all colonies. Connection strength (i.e. line width) between *upper* and *lower boxes* represents the relative volume of a specific pollen morphotype in a colony. Pollen of woody plants is marked with *asterisks*

concentration with pollen weight ($r = 0.43$, $p = 0.017$). Moreover, the proportion of proline correlated positively with colony growth ($r = 0.49$, $p = 0.007$), whereas the essential amino acid lysine was negatively associated with colony growth ($r = -0.51$, $p = 0.004$) (see RDA in Fig. 4b).

Discussion

The 30 *Bombus terrestris* colonies of our study collected pollen from largely similar plant species (dominated by *Acer* and Rosaceae), despite differences in the composition of the surrounding landscape. The number of different pollen morphotypes and the generally low specialization of our colonies agrees with previous studies in other European landscapes (Kleijn and Raemakers 2008; Leonhardt and Blüthgen 2012). However, in contrast to our prediction, pollen diversity did not positively correlate with colony weight gain. Also unexpectedly, colony growth decreased with the proportion of semi-natural habitat in the surrounding landscape and amino acid concentration of stored pollen. Colony growth was, however, positively correlated with total amount of woody pollen and protein content of pollen stored, which agrees with our predictions and previous findings for honeybees (Mattila and Otis 2006) and *B. terrestris* in laboratory trials (Tasei and Aupinel 2008).

Semi-natural habitats typically provide pollen and nectar from a large diversity of vascular plants during the entire foraging season (Scheper et al. 2014; Requier et al. 2015). However, our results suggest that *B. terrestris* does not necessarily forage on these plants early in the season, and that increasing areas of semi-natural habitats may actually correlate with a decrease of areas containing the bumblebees' major pollen sources, i.e. woody habitats. In fact, *B. terrestris* may not rely as much on semi-natural habitats as other, more specialized, bumblebee species, as it is very generalistic in its floral choice and can thus exploit a wide range of habitats including arable fields and urban habitats (Banaszak-Cibicka and Żmihorski 2012; Hanley et al. 2014). This flexible foraging likely explains why only 20 % of pollen was

Table 2 Concentration ± SD (μg/mg) and proportion ± SD (%) of amino acids found in the pollen storage of 30 *Bombus terrestris* colonies experimentally placed at 30 grassland sites

Amino acid	Concentration (μg/mg)	Proportion (%)
Alanine	7.84 ± 17.04	11.3 ± 0.4
Arginine	4.85 ± 7.54	3.5 ± 0.2
Aspartic acid	12.58 ± 20.15	12.1 ± 0.2
Citrulline	0.21 ± 1.66	0.1 ± 0.2
Glutamic acid	11.07 ± 17.9	9.6 ± 0.4
Glycine	6.86 ± 16.87	11.7 ± 0.5
Histidine	2.12 ± 3.34	1.7 ± 0.1
Hydroxyproline	0.59 ± 3.21	0.6 ± 0.4
Isoleucine	2.59 ± 4.78	2.5 ± 0.1
Leucine	9.28 ± 14.33	9.1 ± 0.5
Lysine	5.96 ± 16.18	5.1 ± 1.2
Methamphetamine	0.09 ± 1.85	0.1 ± 0.2
Methionine	1.87 ± 3.67	1.6 ± 0.2
Phenylalanine	4.47 ± 6.85	3.4 ± 0.2
Proline	7.7 ± 12.45	8.7 ± 1.3
Serine	7.14 ± 14.63	8.7 ± 0.4
Threonine	4.42 ± 9.46	4.7 ± 0.4
Tyrosine	2.65 ± 4.34	1.8 ± 0.2
Valine	3.08 ± 6.16	3.3 ± 0.2
β-Alanine	0.15 ± 4.47	0.1 ± 0.4
γ-Aminobutyric acid	0.15 ± 1.84	0.2 ± 0.2

Essential amino acids (following De Groot (1953) for honeybees) are given in *italics*

collected from herbaceous plants, which dominate semi-natural landscapes in the study area. However, Williams et al. (2012) and Requier et al. (2015) suggested that semi-natural habitats likely play an important role later in the season, when floral resources are generally scarce.

Colony growth increased with amount of woody pollen while proportion of forest had no effect. Eighty percent of the pollen volume stored by our colonies came from woody plants. Here, *Acer* was by far the most abundant woody pollen morphotype. Note that our land-use category forest may not necessarily have comprised most of the *Acer* specimens visited by bumblebees for pollen collection, as *Acer* often also occurs in orchards or hedgerows and thus outside of forests, which likely explains the lacking relation with forest area, despite the positive correlation between amount of woody pollen and colony growth. The general importance of woody plants in spring has previously also been shown for honeybees (Odoux et al. 2012; Requier 2013) and wild bees (Bailey et al. 2012). Consequently, in our study, *B. terrestris* appeared to have used *Acer* as a "mass flowering resource" for pollen collection in spring. Our results thus support the hypothesis that *B. terrestris* colonies forage on mass flowering resources when present, as also shown for mass flowering crops (Westphal et al. 2009) and wild plant species (Odoux et al. 2012).

Proportion of urban habitat did neither affect colony growth nor did it correlate with pollen diversity, amount of woody pollen or pollen quality. We had expected a positive relationship with colony growth and pollen diversity because Goulson et al. (2002) suggested that *Bombus terrestris* nests gained weight more quickly, attained a larger final size and stored more diverse pollen if placed in suburban habitats (gardens) rather than farmland. Just like semi-natural landscapes, urban habitats may, however, play an important role for resource allocation later in the season (Scheper et al. 2014; Requier et al. 2015).

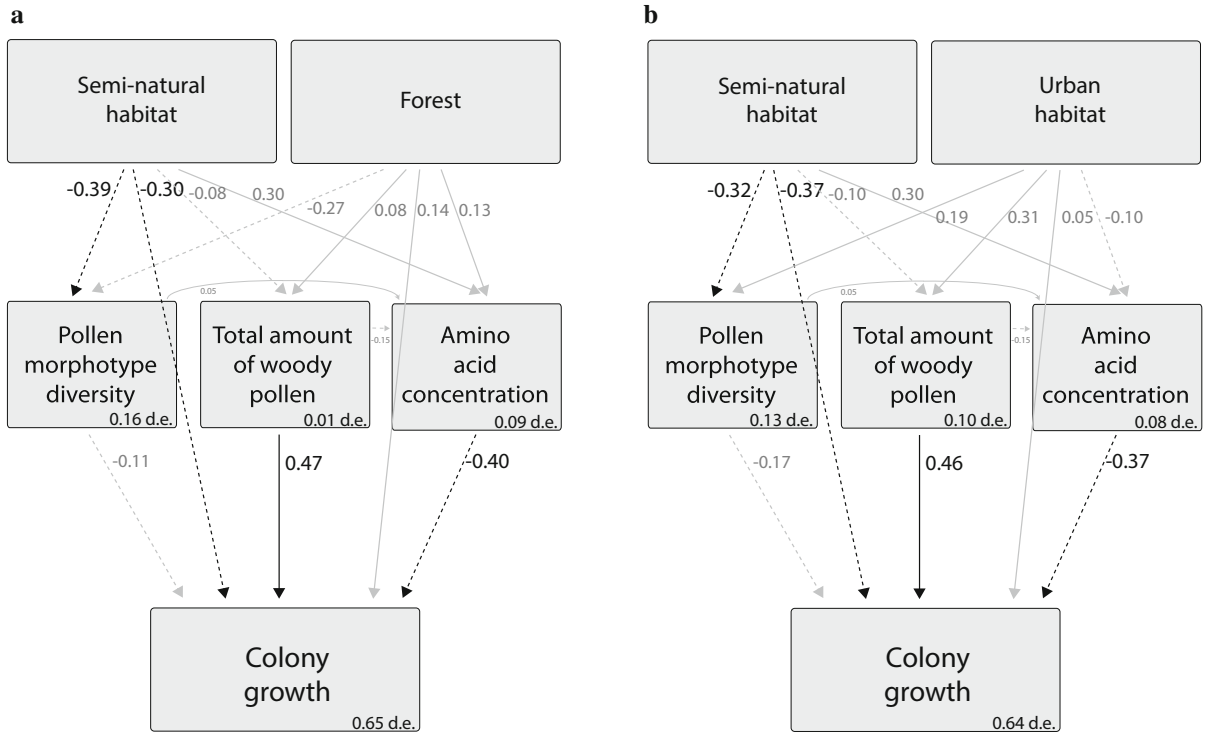

Fig. 3 Piecewise structural equation model (SEM) showing all included interaction pathways. Displayed are direct effects of the sqrt-transformed proportion of (i) semi-natural habitat and (ii) forest (**a**) or urban habitat (**b**) in a 500 m radius around each colony, (iii) pollen morphotype diversity ($e^{H'Pollen}$), (iv) weight of woody pollen in storage, and (v) total amino acid concentration of pollen stored on weight gain of 30 *Bombus terrestris* colonies placed on 30 grassland plots in southwestern Germany in spring, as well as indirect effects of landscape parameters (i and ii) on weight gain via pollen parameters (iii–v). Standardized path coefficient estimates are shown alongside *arrows*. Deviance explained (d.e.) is shown for all endogenous (response) variables next to respective *arrows*. *Solid arrow lines* represent positive interaction pathways, *dashed lines* negative ones. *Black lines* represent significant interaction pathways, *grey lines* non-significant ones

Just like Requier (2013) who found that honeybees visit *Brassica napus* only for nectar, but not for pollen collection, we also did not detect any *B. napus* pollen in any of our colonies. This finding is surprising as *B. napus* was flowering within the 500 m radius of at least six of our colonies, and its pollen has a similar amino acid concentration (38–42 %) as *Acer* (Weiner et al. 2010). However, *B. napus* belongs to the order Brassicales, which produces glucosinolates as secondary metabolites. So far, it has been shown that glucosinolates defend plants against fungal activity (Manici et al. 1997) and herbivory (Wittstock et al. 2003). Whether glucosinolates can be also found in pollen of *B. napus* is unclear, but if so, it may explain why honeybees and bumblebees apparently avoid its pollen.

Our finding that total amino acid concentration of pollen negatively correlated with colony growth disagrees with studies showing that higher protein content in pollen increased bumblebee larval weight (Regali and Rasmont 1995) or the size of *Lasioglossum* offspring (Roulston and Cane 2002). However, when we accounted for the overall amount of pollen stored (by multiplying amino acid concentration with pollen weight), total protein (i.e. sum of all amino acids) stored per colony correlated positively with colony growth, indicating that the overall amount of protein/amino acids is more important for *B. terrestris* colony growth than amino acid concentration *per se*. That is to say, colonies fed with larger amounts of pollen of lower quality gain more weight than colonies fed with lesser amounts of higher quality pollen. This agrees with our predictions and previous findings for honeybees (Mattila and Otis 2006) and *B. terrestris* in laboratory trials (Tasei and Aupinel 2008).

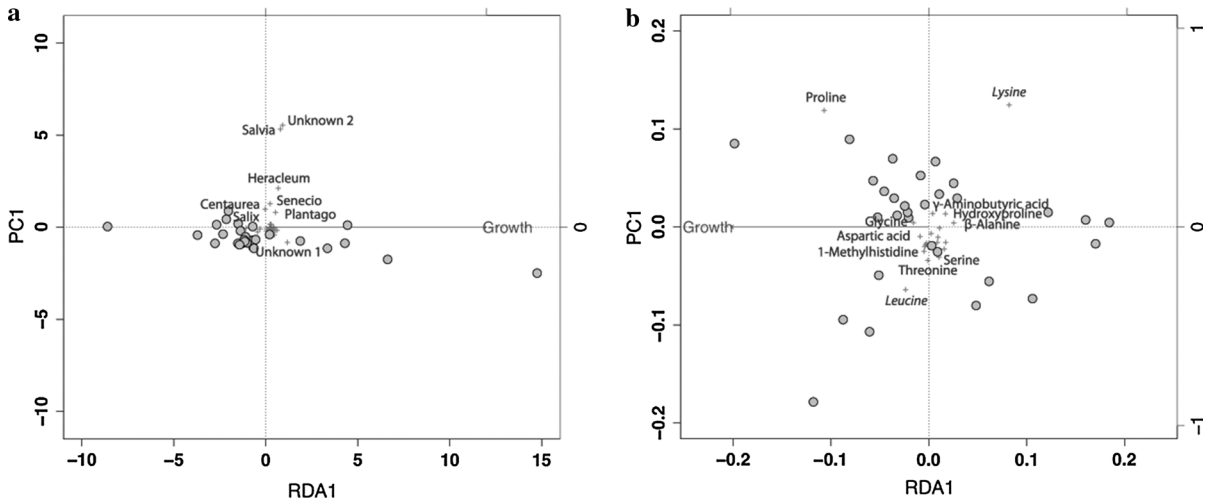

Fig. 4 Visual representation of redundancy analyses (RDA) for proportions of (**a**) pollen morphotype volumes and (**b**) single amino acid concentrations with colony growth fixed as first axis. Essential amino acids are marked in *italics*. *Arrows* point in direction of increasing weight gain. Each *dot* represents one *Bombus terrestris* colony at 30 experimental sites, each cross either **a** a pollen morphotype or **b** an amino acid

Moreover, *B. terrestris* pollen foragers appear to have preferred abundant flowering plant species with average quality pollen over less abundant species with high quality pollen. In doing so, they likely maximized pollen load size and minimized foraging time, which agrees with the optimal foraging hypothesis (Pyke et al. 1977). Efficient foraging is particularly important for bumblebees, because they have one of the highest metabolic costs of flight recorded for any organism (Heinrich 1996). *Bombus terrestris* workers may thus not fly far distances and exclusively collect high quality pollen, but instead maximize pollen intake by collecting from abundant flower patches. Because handling time decreases with experience, collecting pollen from abundant resources is likely to be most efficient and may explain why *B. terrestris* preferentially collects pollen from the abundant woody plant species if available. Maximizing pollen intake may also explain why *B. terrestris* is less likely to mix pollen during a single foraging trip than other bumblebee species (Kratochwil and Kohl 1988; Leonhardt and Blüthgen 2012). For example, *B. pascuorum* workers mixed more pollen types during a foraging trip and also collected pollen from a different plant spectrum. Amino acid concentrations in their pollen were therefore twice as high as in *B. terrestris* (Leonhardt and Blüthgen 2012) suggesting that *B. pascuorum* may maximize quality instead of quantity when foraging on pollen.

Despite differences in total protein/total amino acid content of pollen stores, pollen of all colonies was generally similar in its amino acid composition, indicating that no colony was facing deficiencies in any particular amino acid. This result agrees with the generally balanced amino acid composition found in pollen of most flowering plant species (Weiner et al. 2010). Interestingly, however, colonies with the highest weight gain in our study had disproportionally high amounts of proline and low amounts of lysine in their pollen storage. In honeybees, lysine is an essential amino acid, whereas proline is not. Proline is, however, only semi-dispensable for many insects (Chapman 1998). It is known to have a stimulating effect on larval growth (De Groot 1953) and is important for the flight muscle metabolism in bees (Barker and Lehner 1972; Micheu et al. 2000). Furthermore, proline and lysine have been found to be the most abundant amino acids in royal jelly (Boselli et al. 2003). Lysine is thus certainly important for colony development, but unlike proline may not enhance larval growth when provided in larger quantities. Because all colonies collected the same pollen morphotypes, but amounts of some pollen morphotypes were extremely variable, we could not identify the major plant source of proline.

To sum up, we showed that pollen quantity, not quality, positively correlated with early colony growth in *B. terrestris*, indicating that the availability and abundance of floral resources may be more important in maintaining colony growth than accessibility to plants with high quality pollen. Amount of woody pollen and total protein content was positively correlated with colony growth whereas proportion of semi-natural habitat and amino acid concentration negatively correlated with colony growth. Semi-natural and urban habitats may, however, play an important role for resource allocation later in the season, when floral resource availability is strongly limited (Scheper et al. 2014; Requier et al. 2015). Future studies should comprise additional species, more landscape types and further resource quality parameters (e.g. nectar sugar content, C:N ratios of pollen etc.) to better understand the interaction between landscape, plant community composition, resource availability and quality, and the well-being of pollinators.

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