

# Use of forest strata by bats in temperate forests

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## Keywords

acoustic monitoring; bat community; batcorder; Chiroptera; stratified habitat use.

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## Abstract

Concurrent recordings of bat calls at the ground and canopy levels were compared to analyse the vertical stratification of habitat use in a broad-leaved deciduous forest in central Europe. The recording effort was 48 nights, by sampling 16 sites three times in 2010. Overall, 2170 call sequences were recorded; 40% of them at the canopy level. Sixteen bat species were identified, 13 of which occurred at ground level and 14 in the canopy. By fitting generalized linear mixed models for seven species and all calls sequences combined, the effects of stratum, time period, ambient temperature and forest structure on bat-calling activity were assessed. Four species preferred the ground level (*Barbastella barbastellus*, *Eptesicus serotinus*, *Myotis daubentonii*, *Pipistrellus pygmaeus*). *M. bechsteinii* was the only species which had a significant preference for the canopy level in the pregnancy, lactation and post-lactation period, whereas two further species (*M. alcaethoe*, *P. pipistrellus*) showed a significant canopy preference in at least one period. Therefore, canopy sampling should be undertaken when aiming at monitoring such species for conservation purposes. Populations of certain target species will be underestimated by ground recordings alone.

## Introduction

Habitat selection by bats in temperate forests had been the focus of several studies (Kanuch *et al.*, 2008; Napal *et al.*, 2010). Because of its spatial extent, the forest interior is the most important foraging habitat, which also harbours the highest bat species diversity (Celuch & Kropil, 2008). Numerous studies on vertical stratification within forests are available on bats from tropical ecosystems, stimulated by the complex vertical structure and species composition in rainforests (e.g. Henry *et al.*, 2004; Kalko *et al.*, 2008; Pereira, Marques & Palmeirim, 2010). All these studies show differences in the abundance and species composition between the understory level and the canopy stratum. Therefore, many bat species can be assigned to a specific stratum, whereas others forage through all forest strata. In temperate-zone forests (North-America: Bradshaw, 1996; Wunder & Carey, 1996; Kalcounis *et al.*, 1999; Hayes & Gruver, 2000; Australia: Adams, Law & French, 2009; Europe: Collins & Jones, 2009; Zeus, 2010), the canopy is an important foraging habitat where overall activity is similar to the understory. In North-American and European forests, bat species composition appears to be similar between the strata, but there are significant differences in the activity level of certain species and species groups. However, most studies suffer from insufficient species discrimination (e.g. in the genus *Myotis*) or small sample sizes.

The aim of the present study was to assess the stratification of bats in a forest region in central Europe by means of automated acoustic monitoring, which is at least as effective as mist-netting in regard to surveying bat community structures (O'Farrell & Gannon, 1999; MacSwiney, Clarke & Racey, 2008). Of course, acoustic surveys are biased because some echolocation calls are more readily detected than others. This depends on the call intensity and the varying sensitivity on the microphones to certain frequencies. Comparative acoustic surveys can be valuable as biases in detectability of calls between strata are probably negligible. To assess the influence of the stratum on the foraging activity, it is necessary to include the most important forest parameters and the temperature in the calculated models. The forest structure is strongly linked to the bat species present (Ford *et al.*, 2005) and temperature can be used as a predictor for insect activity (Dajoz, 2000). For some bats, like ground gleaning and aerial foraging species, a clear stratification was expected. In contrast, the life habits of other species are so poorly known that habitat use cannot be predicted. We tested the following predictions:

- A clear stratification for ground gleaning bats and open space species will occur because of their foraging behaviour, whereas bats that hunt close to the vegetation will use both strata equally.
- Bat species that forage in both strata can change their preference according to their reproduction cycle because lactating

and post-lactating females are more manoeuvrable than pregnant females in the dense canopy stratum.

## Materials and methods

### Recording and analysis of bat calls

For recording bat calls, we used two automated ultrasound recording units of the same type ('batcorder', ecoObs, Nuremberg, Germany, <http://www.ecoobs.de>). Batcorders digitally record ultrasonic signals in real time (500 kHz, 16 bit) and use an online analysis to distinguish between bat calls and ultrasound signals from other origins (e.g. bush crickets). Further strengths of this system are the comparability of the results between different devices (calibrated sensitivity) and the omnidirectionality of the microphone. This system was originally designed for studies on microhabitat use by bats in forests (Runkel, 2008). Devices were calibrated and configured by ecoObs. No further adjustments of the settings were made (400 ms post-trigger, -27 dB threshold level). The timers of the batcorders were set to record the full night (2 h before sunset until 2 h after sunrise). The recording sensitivity of the devices was regularly checked.

Recorded bat calls were automatically stored and measured with the software bcAdmin 2.06 (ecoObs). Then, measurements were analysed with the software batIdent 1.02 (ecoObs). This software compares unknown bat calls with reference calls based on a mathematical approach (package 'Random forest' in R). This system is able to achieve 95% correct classifications under training conditions. In the field, these conditions are rarely met and some identifications made here – especially *Myotis* species like *Myotis bechsteinii* with very similar calls – may be incorrect (Marckmann & Runkel, 2010). We emphasize that our study is a comparative one and we see no reason to suspect differences in the likelihood of correct classification between the two strata. After this automated classification, each sequence was manually checked using bcAnalyze 1.11 (ecoObs) and published descriptions for bat calls of the different species (Zingg, 1990; Russo & Jones, 2002; Obrist, Boesch & Flückinger, 2004; Hammer, Zahn & Marckmann, 2009). Each sequence that was classified as 'Nyctaloid' (*Nyctalus* sp., *Eptesicus* sp., *Vespertilio murinus*) was also manually checked with BatSound 4.1 (Pettersson Elektronik AB, Uppsala, Sweden) using a discrimination function (Zingg, 1990; Sattler *et al.*, 2007). Collectively, these procedures resulted in a manual classification of each call sequence to the lowest possible operational taxonomic unit (OTU).

### Study area and data collection

This study was conducted in 2010 in the Thayatal National Park in northern Austria. There, three forest types are dominant: beech (*Fagus sylvatica*), hornbeam (*Carpinus betulus*) and oak forests (*Quercus* sp.). An inventory study of bats by Hüttmeir, Reiter & Reiter (2011) recorded 20 bat species for this study area. Therefore, it was suitable to assess the stratification by bats, because a wide range of species was expected.

We selected 16 sampling sites in the study area. The minimum distances between two sites ranged from 610 to 1690 m (mean 967 m). Each site was selected to be characteristic for the surrounding forest. No water bodies, forest edges, forest roads or other possible flyways were located within 50 m of the sampling site, ensuring that we were investigating the forest interior and not ecotones.

Batcorders were put in place following Humphrey, Bridge & Lovejoy (1968), Munn (1991) and Kunz & Parsons (2009). By using a slingshot, ropes were placed as close to the upper canopy as possible. According to the different forest heights at the sampling sites, the height of the upper batcorder varied from 10.0 to 19.0 m [mean height: 13.9 m, standard deviation (SD) = 2.1 m]. The batcorder at the ground level was mounted at a height of 2.30 m. Each measurement night, batcorders used for the different strata were switched to minimize bias because of possible sensitivity differences between units.

At each site, recordings were made during three time periods. Period 1 covered May and June and comprised the pregnancy phase. Period 2 included July and August during the lactation phase and Period 3 was in September during the post-lactation and migration phase.

In addition to the recording of bat calls, 19 structural habitat parameters (Supporting Information Table S1) of the forest were measured (Ford *et al.*, 2005; Kanuch *et al.*, 2008). Furthermore, an iButton temperature logger (SL52T, precision  $\pm 0.5^\circ\text{C}$ , Signatrol Ltd, Tewkesbury, UK, <http://www.signatrol.com>) was mounted at each batcorder. Data loggers were configured to record ambient temperature every 15 min.

### Statistical analyses

As a first step, a species  $\times$  sampling point matrix was created, with the number of sequences in each cell. For identification of the most important structural habitat parameters for further analysis, this matrix was converted into a standardized Bray–Curtis distance matrix using PRIMER-E (Clarke & Gorley, 2006). With this matrix, a non-linear multidimensional scaling (NMDS) was performed. For this purpose, the three replicate bat surveys for each site were pooled to achieve a more complete local species abundance list. In the resulting NMDS ordination plot, bat communities at the 16 sampling sites were distributed along the first ordination axis. No obvious clustering of different forest types (e.g. according to the dominant tree species) and no stratification with regard to bat species activity was detected. The first and second ordination axis values were then extracted from the NMDS for each site, and Pearson correlations of these site scores in reduced ordination space with the standardized habitat parameters from the field survey were calculated. To achieve normality of habitat variables, we transformed them as necessary. All parameters that were significantly correlated with the position of the bat communities in reduced ordination space were identified as important and were thus considered for further analyses. The first ordination axis of the NMDS plot was mainly correlated with forest structure parameters. Many of these parameters were multicollinear to each other; therefore, a principal component

analysis (PCA) was performed. The first and second principal components of this PCA were then used for further analysis. All analyses mentioned earlier were conducted with the software Statistica 9 (StatSoft GmbH, Hamburg, Germany). We calculated mean nightly temperature although for three nights, no temperature data were available because of logger failure. We used a linear regression of the other recorded nights to the temperature data of the nearest weather station in Retz to estimate missing mean temperatures.

To analyse the effects of stratum, period and the most important habitat parameters, generalized linear mixed models (GLMM) were calculated with R 2.12.0 (R Development Core Team, 2010) using the package lme4 (Bates, 2005). For each bat species (or OTU) with more than 30 call sequences recorded, a GLMM was fitted. In addition to this, a GLMM was fitted for all sequences of all species combined to show overall effects of stratum and period on calling activity among Chiroptera. All OTUs with indistinguishable call sequences were excluded from further analyses because different, acoustically inseparable species are united within these OTUs. For each GLMM, the dependent variable was the number of call sequences for the respective species in each night and batcorder ( $n = 96$ ), the independent variables were stratum (ground or canopy), period (1, 2 or 3), mean temperature (z-standardized), PCA1 and PCA2 of the habitat variables, and the interaction term between stratum and period. Sampling site ( $n = 16$ ) and the recording night ( $n = 48$ ) were used as random factors. For the GLMMs, a Poisson distribu-

tion as family function and a log link were used. After the full GLMM had been calculated for each species, a stepwise backward reduction was performed. If the interaction of stratum and period was not significant, it was dropped. In the next steps, the parameter with the highest  $P$ -value was removed ( $P$ -value at reduction point is always listed) and a reduced model was fitted. The GLMM was further reduced until only parameters with a  $P$ -value  $< 0.05$  were included. In this model building approach, no Bonferroni correction was adopted (Moran, 2003); therefore, significance levels should not be interpreted like tests of *a priori* hypotheses (Forstmeier & Schielzeth, 2011). Since the package lme4 offers no single  $P$ -value for factors with more than two levels, it is listed in the table if at least one value for period was significant. If the interaction of stratum and period was significant, the effect of stratum was analysed by re-calculating the final GLMM with subsets for each period. Furthermore, we indicate where bat activity differed significantly between strata in at least one period. Because our data might be overdispersed and the significance therefore somewhat overestimated, we interpreted clear effects as those with a  $P$ -value  $< 0.03$ .

## Results

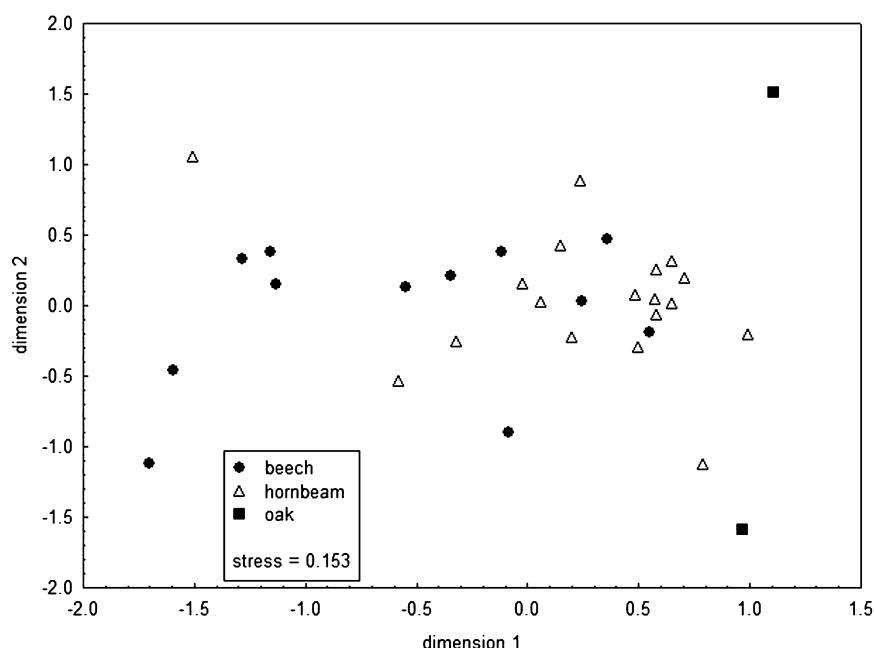
We recorded 2170 call sequences in 48 nights (Table 1). Sixty-five per cent of all calls were assigned to the lowest possible level. In most cases, this was the species level, except for *M. brandtii* and *M. mystacinus*, which were assigned to an OTU

**Table 1** Numbers of recorded call sequences per OTU

OTU	Period 1		Period 2		Period 3		Overall		Sum
	G	C	G	C	G	C	G	C	
<b><i>Pipistrellus pipistrellus</i></b>	481	26	35	100	52	56	568	182	<b>750</b>
Mkm	128	89	75	88	9	57	212	234	<b>446</b>
<i>Myotis sp.</i>	94	55	34	66	0	30	128	151	<b>279</b>
Mbart	70	25	36	36	6	19	112	80	<b>192</b>
<b><i>Myotis alcathoe</i></b>	23	25	6	35	6	31	35	91	<b>126</b>
<b><i>Pipistrellus pygmaeus</i></b>	6	5	1	3	57	22	64	30	<b>94</b>
<b><i>Myotis daubentonii</i></b>	43	10	11	2	0	0	54	12	<b>66</b>
<b><i>Myotis bechsteinii</i></b>	14	25	6	11	0	4	20	40	<b>60</b>
<b><i>Barbastella barbastellus</i></b>	8	1	21	9	9	3	38	13	<b>51</b>
<b><i>Eptesicus serotinus</i></b>	3	1	23	4	0	0	26	5	<b>31</b>
Chiroptera sp. indet.	3	2	7	10	4	4	14	16	<b>30</b>
<i>Myotis myotis</i>	5	0	5	0	3	0	13	0	<b>13</b>
<i>Myotis emarginatus</i>	4	1	0	1	0	3	4	5	<b>9</b>
<i>Myotis nattereri</i>	0	0	3	0	4	0	7	0	<b>7</b>
<i>Eptesicus nilssonii</i>	0	1	5	0	0	0	5	1	<b>6</b>
<i>Nyctalus leisleri</i>	0	3	1	0	0	1	1	4	<b>5</b>
<i>Rhinolophus hipposideros</i>	0	0	0	0	0	2	0	2	<b>2</b>
<i>Nyctalus noctula</i>	0	0	0	2	0	0	0	2	<b>2</b>
<i>Vespertilio murinus</i>	0	0	0	0	0	1	0	1	<b>1</b>
Sum	882	269	269	367	150	230	1301	869	
	<b>1151</b>		<b>636</b>		<b>380</b>		<b>2170</b>		

Only for OTUs in bold letters were further analyses performed. Mbart consist of *Myotis brandtii* and *M. mystacinus*. Mkm is an OTU of calls of *M. alcathoe*, *M. daubentonii*, *M. bechsteinii* and Mbart.

OTU, operational taxonomic unit; G, ground level; C, canopy level.



**Figure 1** Non-linear multidimensional scaling ordination plot of bat communities based on Bray–Curtis distance matrix of call sequences. Each dot depicts one site, divided in ground or canopy stratum. The forest types did not clearly segregate from each other.

termed Mbart. The percentage of undetermined sequences was higher in the canopy stratum (46.1%) than near the ground (27.2%). We checked recordings for overlapping calls between both strata and only 4.4% were recorded in the same time interval ( $\pm 1$  min).

The mean nightly temperature during the first period was  $12.4^{\circ}\text{C}$  ( $\pm 2.63$  SD) and  $16.4^{\circ}\text{C}$  ( $\pm 2.33$  SD) in the second period. The third period was the coldest, with a mean temperature of  $10.5^{\circ}\text{C}$  ( $\pm 2.78$  SD). Between the two strata, there was no difference in the mean temperature (paired  $t$ -test:  $t = -0.86$ ,  $P = 0.39$ , degrees of freedom = 47).

Altogether, we detected 16 bat species (including Mbart; Table 1). Of these, 13 were found at the ground level, with the exception of *Rhinolophus hipposideros*, *Nyctalus noctula* and *Vespertilio murinus*. In contrast, *M. myotis* and *M. nattereri* were never recorded at canopy level.

The first ordination axis of the NMDS (Fig. 1) was significantly correlated with various habitat parameters. The second ordination of the NMDS correlated only weakly with the height of the batcorder (Supporting Information Table S1). In a PCA for the habitat parameters identified as important by the NMDS, the first principal component (PCA 1) described 56.04% of the variance, the second principal component (PCA 2) 16.41%, while the third accounted for less than 10%. PCA 1 was mainly composed of tree-height, variance of the breast-height diameter, basal tree area, density of the ground related stratum, large tree trunks and the distance to the next forest edge (factor loadings in this order). All bats combined, *Pipistrellus pipistrellus* and *M. daubentonii* were the only OTUs that showed a significant response in activity to PCA 1 (Table 2). At sites where the forest was denser and the amount of obsta-

cles increased, their calling activity declined. In contrast PCA 2, which was composed of the distance to the next settlement and the amount of large tree trunks, was significant for Mbart. Activity increased with the distance to the next settlement and the amount of large tree trunks.

For all eight OTUs analysed (Table 2), a significant overall effect of stratum was found with the GLMMs, calling activity was not evenly distributed between canopy and understory. Over the three periods, a significant difference in the calling activity was found for six OTUs. Also, the significant interaction of period  $\times$  stratum shows that calling activity varied across space and time for all bats combined and for the OTUs *M. alcaethoe* and *Pipistrellus pipistrellus*. The forest structure, measured as PCA 1 and PCA 2, had a significant influence on call numbers only for all bats combined, *M. daubentonii* and *P. pipistrellus*.

*Barbastella barbastellus*, *Eptesicus serotinus*, *M. daubentonii* and *Pipistrellus pygmaeus* were significantly more active near ground level compared with the canopy while *M. bechsteinii* exhibited a significantly higher activity in the canopy stratum (Fig. 2).

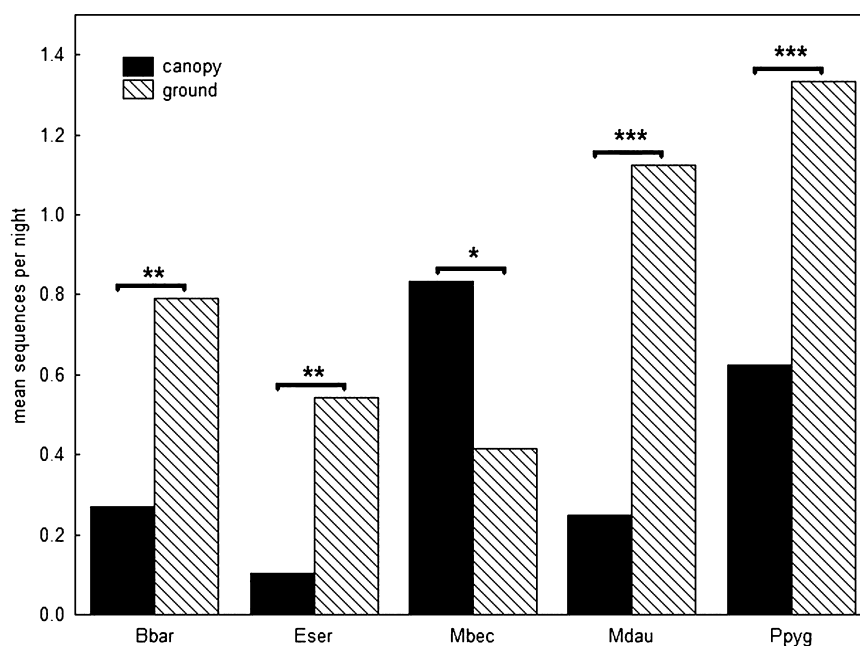
Bats, in general (Fig. 3), showed a significantly higher activity in the understory in the pregnancy period ( $z = -17.05$ ,  $P < 0.001$ ). During lactation ( $z = 3.87$ ,  $P < 0.001$ ), and in the post-lactation and migration periods ( $z = 4.20$ ,  $P < 0.001$ ), bat activity in the canopy stratum was higher. *M. alcaethoe* (Fig. 4) showed significant stratification in periods 2 ( $z = 3.92$ ,  $P < 0.001$ ) and 3 ( $z = 3.61$ ,  $P < 0.001$ ), with a strong preference for the canopy stratum. In period 1 ( $z = 0.28$ ,  $P = 0.78$ ) no stratification occurred. *P. pipistrellus* (Fig. 5), exhibited a significant preference for the understory level in period 1 ( $z = -14.48$ ,

**Table 2** Results of the final generalized linear mixed models

Species	Stratum		Temperature		PCA 1		PCA 2		Period	Period × stratum
	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>		
All		<b>s.</b>	0.34	0.74	2.03	<b>0.043</b>	1.75	0.08	<b>s.</b>	<b>s.</b>
Bbar	−3.19	<b>0.001</b>	0.62	0.54	0.49	0.62	1.08	0.28	n.s.	n.s.
Eser	−3.30	<b>0.001</b>	1.35	0.18	0.70	0.48	0.03	0.98	n.s.	n.s.
Malc		<b>s.</b>	1.98	<b>0.047</b>	1.41	0.16	0.89	0.37	<b>s.</b>	<b>s.</b>
Mbec	2.44	<b>0.015</b>	0.33	0.74	0.83	0.41	0.07	0.95	<b>s.</b>	n.s.
Mdau	−4.66	<b>&lt;0.001</b>	1.84	0.66	2.99	<b>0.003</b>	0.75	0.45	<b>s.</b>	n.s.
Ppip		<b>s.</b>	0.51	0.61	2.30	<b>0.021</b>	0.13	0.90	<b>s.</b>	<b>s.</b>
Ppyg	−3.40	<b>&lt;0.001</b>	0.08	0.94	−0.33	0.74	0.09	0.93	n.s.	n.s.

Given are *z*- and *P*-values for the variables included in the models. *s* indicates that a least one factor was significant, otherwise it is marked as n.s. For models with a significant interaction, no single *P*-value for stratum can be shown.

PCA, principal component analyses; All, all bats combined; Bbar, *Barbastella barbastellus*; Eser, *Eptesicus serotinus*; Malc, *Myotis alcathoe*; Mbec, *Myotis bechsteinii*; Mdau, *Myotis daubentonii*; Ppip, *Pipistrellus pipistrellus*; Ppyg, *Pipistrellus pygmaeus*.



**Figure 2** Number of sequences per night (averaged over all three periods) at the canopy and ground level. See Table 2 for abbreviations. \* = 0.05–0.01, \*\* = 0.01–0.001, \*\*\* = <0.001.

$P < 0.001$ ), in period 2 a significantly higher activity in the canopy stratum ( $z = 5.32$ ,  $P < 0.001$ ) and no difference in period 3 ( $z = 0.38$ ,  $P = 0.70$ ).

## Discussion

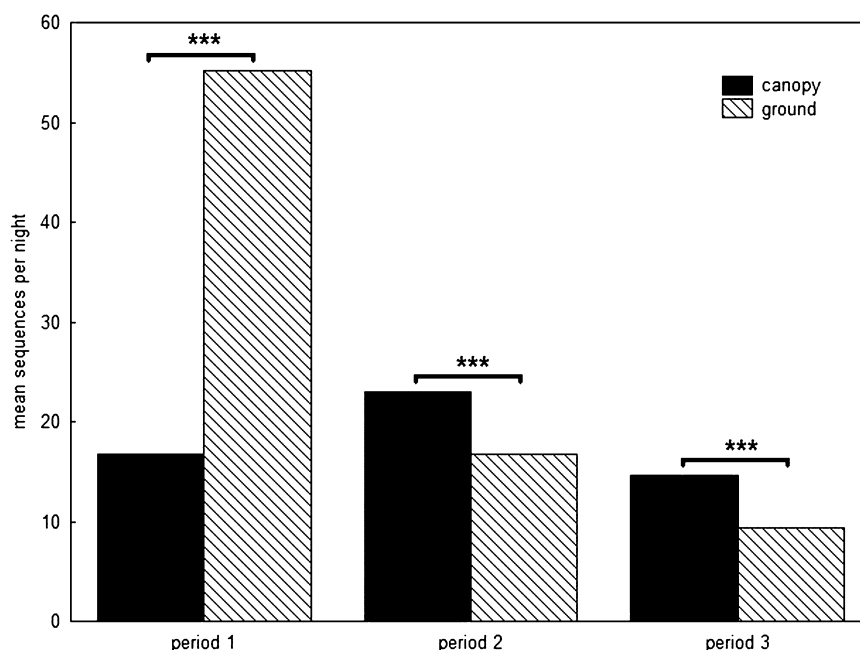
### Effect of the forest stratum on bat activity

Our study emphasizes the importance of the canopy stratum as a foraging habitat of bats. As in similar studies (Kalcounis *et al.*, 1999; Collins & Jones, 2009; Zeus, 2010), in the northern hemisphere, and in contrast to many tropical forests, no clear stratification of species composition emerged. The species that

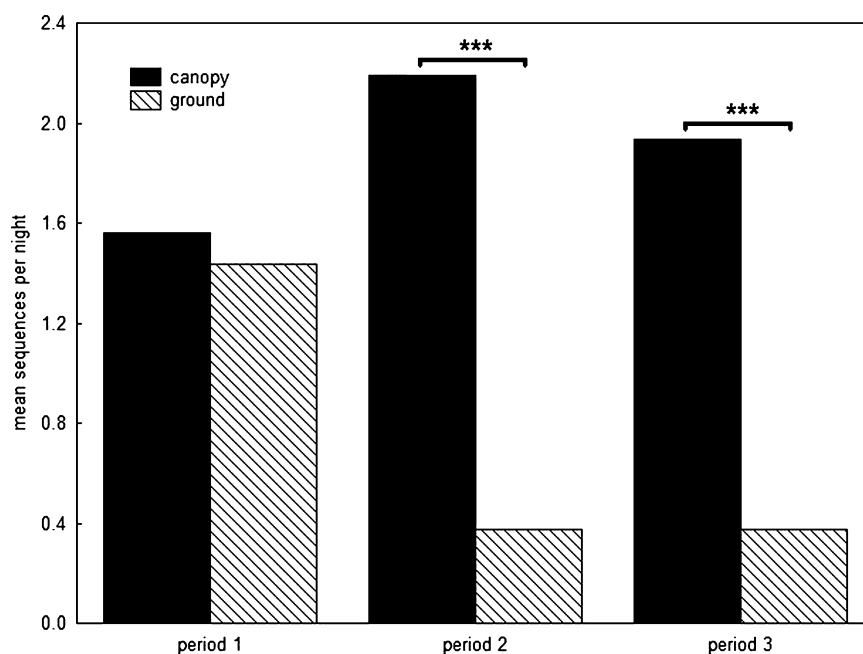
occurred in just one stratum accounted for 1.2% of all call sequences; therefore, their habitat selection are possibly because of sampling effects and no further analyses were conducted. For some of these, for example *M. myotis*, a clear stratum preference was expected. In addition, we found complex spatio-temporal activity patterns for the more commonly detected species.

The combined data for all bats emphasize the importance of the canopy stratum. While in spring and early summer, the activity in the understory was significantly higher, the canopy stratum became more important later in the season. This stratum shift may depend on variation in insect availability, which is affecting both male and females or on differential





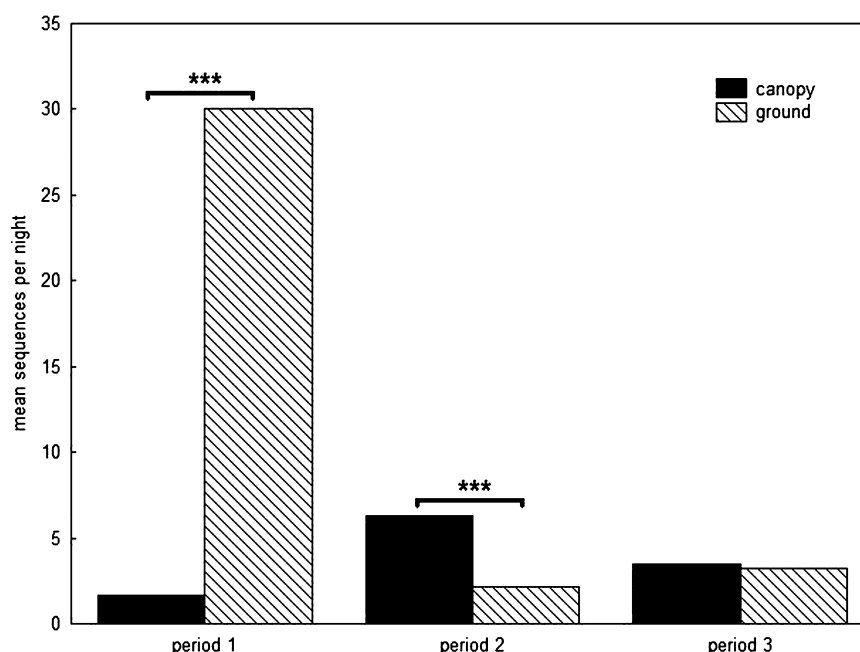
**Figure 3** Number of sequences per night (averaged over all three periods) at the canopy and ground level across all three periods for all bats combined. \* = 0.05–0.01, \*\* = 0.01–0.001, \*\*\* ≤ 0.001.



**Figure 4** Number of sequences per night (averaged over all three periods) at the canopy and ground level across all three periods for *Myotis alcathoe*. \* = 0.05–0.01, \*\* = 0.01–0.001, \*\*\* ≤ 0.001.

ability of female bats to exploit the canopy stratum during pregnancy. The analysis of data for individual species revealed more complex patterns. Some species showed a clear stratification in all three periods, with a preference for the ground or

the canopy, respectively. Also two species switched their foraging habitat preference during the year. Apparently every species has unique habitat preferences, so an evaluation at the genus level would be misleading. This is exemplified by the



**Figure 5** Number of sequences per night (averaged over all three periods) at the canopy and ground level in all periods for *Pipistrellus pipistrellus*. \* = 0.05–0.01, \*\* = 0.01–0.001, \*\*\* ≤ 0.001.

genus *Myotis* which consists of six species in the study region, all with distinctive foraging strategies.

Even species that had a significantly higher activity at the ground level were regularly found in the canopy stratum. About 75% of all calls from *B. barbastellus* were recorded at ground level. This species typically inhabits woodlands of all kinds, with a preference for highly structured habitats. Radio-tracked individuals were described to forage close to the vegetation and above the forest canopy (Sierro & Arlettaz, 1997). Zeus (2010) also observed the main activity of *B. barbastellus* near the ground. These data suggest that *B. barbastellus* generally prefers the ground level of the forest over the canopy stratum. *E. serotinus* also significantly preferred the ground level. The temporal distribution of call sequences shows that 65.4% of the records at ground level occurred from 20.30 to 21.30. This suggests that *E. serotinus* uses the more open space close to the ground for commuting from its roosting sites to the feeding grounds outside the forest. *M. daubentonii* had exhibited a significant preference for ground level. Its decreased activity in denser forest sites indicates that it prefers open forest patches at ground level and avoids the dense canopy stratum. A reason for this might be that it is not able to detect prey as successfully in front of a background compared with other *Myotis* species (Siemers & Schnitzler, 2004). *P. pygmaeus*, had also a higher activity at the ground level than in the canopy stratum across all three periods. It is known to be hunting closer to the vegetation and at lower heights in contrast to *P. pipistrellus* (Arnold, Häussler & Braun, 2003; Collins & Jones, 2009). Our study also indicates that *P. pygmaeus* has a different habitat preference in the forest interior, where both *Pipistrellus* species co-occur. At

least in the second and third period, the main activity of *P. pipistrellus* was not at the ground level.

Only one species was significantly more active in the canopy stratum across all three periods. *M. bechsteinii* is restricted to woodlands, especially to old growth broadleaved forests (Dietz & Pir, 2009; Zahn *et al.*, 2010), where it prefers the interior of the forest with high canopy cover (Napal *et al.*, 2010). Therefore, the Thayatal National Park is a suitable habitat for this species, which is a priority for conservation (Hüttmeir *et al.*, 2011). According to Dietz, Helversen & Nill (2009), *M. bechsteinii* forages close to the vegetation. Depending on the habitat structure, it flies close to the ground or in the canopy stratum. It uses prey-generated sounds to detect prey hidden in the vegetation (Siemers & Swift, 2006). Our study indicates that it has a clear preference for the canopy area, possibly depending on the selected uniform forest sites without open patches, where the cover of the shrub layer was very low.

Two species had a varying preference for the stratum during the year. *M. alcathoe* was only described 10 years ago (von Helversen *et al.*, 2001). No data have yet been published about vertical stratification by *M. alcathoe*, but it is known to use the lower canopy of broadleaved trees (Dietz *et al.*, 2009). The roosting sites of *M. alcathoe* seem to be fissures or small cavities in the canopy, up to 16 m above ground (Dietz *et al.*, 2009; Lucan *et al.*, 2009). We found a clear stratification in the second and third period (Fig. 4). Therefore, we conclude that *M. alcathoe* has a preference for the canopy stratum and only during the pregnancy period a meaningful proportion of the activity was on the ground stratum, because of the reduced agility of the females. *P. pipistrellus* is common in Europe (Dietz *et al.*, 2009) and uses a variety of habitats (e.g. Oakeley

& Jones, 1998; Sattler *et al.*, 2007). In the first period, a significant preference for the ground level was recorded. However, in the second and third periods, the habitat preference varied from the first period. Also, in the study of Zeus (2010), a switch in the usage of the strata was found. In consideration of the flexibility in the selection of the habitat and their broad range of prey species (Arnold *et al.*, 2003; Dietz *et al.*, 2009), it seems they are very variable in the lactation and post-lactation period. Other parameters like interspecific competition with other species like *P. pygmaeus* may also be important.

## Conclusion

For conservation-oriented inventory studies, it is important to detect all species that occur. During the acoustic survey from the ground, similar species richness was recorded compared with the canopy stratum. Because of the logistics, we do not recommend to include canopy recordings in simple inventories of bat species. However, when monitoring not only species presences, but also activity (as a proxy for abundances), the stratification of bat activity should be considered. In our study, 50% of the analysed species showed a significantly higher activity in the canopy in at least one period. Depending on the species that are monitored, it is recommended to consider recordings also in the canopy level.

For conservation measures in the European Union (EU), endangered species and species of EU-wide importance are most relevant. We recorded at least 16 species that are listed in the annex IV and five species of the annex II of the habitats directive (92/43/EEC). For two of the annex II species, we performed a detailed analyses. *B. barbastellus* showed a higher activity at the ground level. Because of contrasting findings in other studies, further research is needed to resolve these discrepancies. *M. bechsteinii* is an annex II species that had a higher activity in the canopy stratum compared with the ground level. Because it is a specialized forest species (Meschede & Heller, 2002), which has its main activity in the interior of near-natural woodlands, our findings are important. Our results are also important to inform management plans, especially in working forests. If specialized and endangered forest species are monitored only from the ground, their conservation status would be estimated on biased data.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Full list of all habitat variables recorded. The columns NMDS1 and NMDS2 give the correlation values to the first and second ordination axis of the NDMS (Fig. 1).

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