Composition of guano produced by frugivorous, sanguivorous, and insectivorous bats

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Bat guano supports an assemblage of organisms that varies depending on the species of bat producing it. To determine whether these differences in community structure may be due to differences in guano composition, we analyzed guano from frugivorous (*Pteropus rodricensis*), sanguivorous (*Desmodus rotundus*), and insectivorous (*Tadarida brasiliensis*) bats. We found no differences among species in organic matter or lipid of guano. *Desmodus* guano contained more carbon (C) than *Pteropus* guano. The latter contained less nitrogen (N), and the former contained less phosphorous (P) than guano of the other two species. *Pteropus* guano had a higher C:N ratio, and *Desmodus* guano had higher N:P and C:P ratios than the other two species. These differences in guano composition suggest that guano from bats in different feeding guilds may affect ecosystem structure and dynamics differently.

Key words: Chiroptera, frugivorous, insectivorous, sanguivorous, guano, composition, carbon, nitrogen, phosphorus, stoichiometry

Introduction

Connectivity between communities is maintained by the redistribution of biomass, often by mobile animals that consume resources in one habitat and subsequently reproduce, urinate, and/or defecate in other habitats. This transfer of organic material affects the nutrient budget of a community and influences population and food web dynamics. In terrestrial habitats, mobile animals such as mammalian herbivores (McNaughton, 1985) and birds (Weir, 1969) transport substantial quantities of biomass between communities. Allochthonous input of nutrients such as nitrogen and phosphorus, which are found in relatively high concentrations in bird guano, increases primary productivity in terrestrial ecosystems by enhancing the quality and quantity of vegetation (see Polis *et al*., 1997). Nutrient input through guano deposition by seabirds has also been shown to increase the abundance of organisms such as detritivorous beetles on islands used by roosting seabirds (Sánchez-Piñero and Polis, 2000).

In addition to its effects on primary and secondary productivity, allochthonous nutrient input can also influence community structure. For example, the presence of birds and nutrient-rich guano significantly alters the structure of intertidal communities by enhancing algal growth and settlement of invertebrates in dense algal mats (Bosman and Hockey, 1986). Such consumer-driven nutrient recycling via

fecal deposition by bats also affects community structure in guano-based ecosystems. Bat guano forms the basis of a food web consisting of bacteria, fungi, protozoans, nematodes, and arthropods (Harris, 1970). Fenolio *et al.* (2006) even demonstrated that cave salamanders consume guano of grey bats (*Myotis grisescens*) and incorporate the nutrients they obtain through coprophagy into body tissues. The diversity of organisms associated with guano has been shown to vary depending on the diet of the bat producing it, with guano of sanguivorous, insectivorous, and frugivorous bats supporting different assemblages of invertebrates (Ferreira and Martins, 1998).

Given that the suites of consumers associated with guano differ depending on the dietary strategy of the bat, we suggest that these differences may result from differences in nutrient content among guano types. Although several studies have compared the effects of guano from different bat species on community structure and nutrient cycling (Ferreira and Martins, 1998, 1999; Shahack-Gross *et al.*, 2004), the composition of guano from bats consuming different diets has received little attention. Because of the importance of bat guano in food web dynamics and the observed differences in community structure supported by various types of guano, we wanted to determine if these differences in guano pile communities may be attributable to differences in the chemical components of guano. We therefore evaluated the composition of guano from three species of bats with different dietary habits: frugivory (*Pteropus rodricensis*), sanguivory (*Desmodus rotundus*), and insectivory (*Tadarida brasiliensis*).

MATERIALS AND METHODS

Bat guano was collected from three locations for each of three bat species. *Pteropus rodricensis* (family Pteropodidae) is a frugivorous bat species native to Rodriguez Island in the Indian Ocean. This Old World fruit bat typically roosts in trees of forests and swamps and consumes a diet of fruit juices and some soft fruit pulp (e.g., bananas) in the wild. *Desmodus rotundus* (family Phyllostomidae) is a sanguivorous bat species native to Central and South America. This American leaf-nosed bat typically lives in caves, hollow trees, and abandoned buildings and primarily feeds on the blood of horses, cows, and pigs. *Tadarida brasiliensis* (family Molossidae) is an insectivorous species found from the southern United States to northern South America (Nowak, 1999). The subspecies found in Florida, *T. brasiliensis cynocephala*, is neither migratory nor cave-dwelling, unlike the Mexican subspecies (*T. brasiliensis mexicana* — McCracken and Gassel, 1997). We chose these three species for our study based on their diets and because of their accessibility in American zoos (*P. rodricensis* and *D. rotundus*) or in the wild in Florida (*T. brasiliensis*).

Fresh guano from captive *P. rodricensis* was collected by staff of the Philadelphia Zoo (Philadelphia, PA) and the Cleveland Metroparks Zoo (Cleveland, OH) and by us at the Lubee Foundation in Gainesville, Florida. These facilities feed their *P. rodricensis* a diet consisting of mixed fruits and vitamin supplements. Fresh guano from captive *D. rotundus* was collected by staff of the Sedgwick County Zoo (Wichita, KS), the Houston Zoo (Houston, TX), and the North Carolina Zoo (Asheboro, NC). These bats were fed a diet consisting of bovine blood with an anticoagulant. We collected fresh guano from wild *T. brasiliensis* from two roosts in bridges of the Florida Turnpike (at overpasses for County Route 561 and State Route 438) in central Florida. We also obtained a sample of guano from wild *T. brasiliensis* harvested previously from the University of Florida bat house by staff of the University's Environmental Health and Safety Office. This bat house is home to a colony consisting largely of *T. brasiliensis*, although some insectivorous southeastern bats (*Myotis austroriparius*) share the roosts. Because each of our *T. brasiliensis* guano samples was collected from wild bat colonies, we assume the diet of these bats consisted of a variety of flying insects (e.g., moths and beetles — Nowak, 1999) found in Florida.

To collect each fresh guano sample, plastic tarps were placed on the floor of the bat enclosure (or on the ground for the two *T. brasiliensis* samples collected from overpasses) for a minimum of two hours on each of six consecutive days to collect guano from bats that had recently eaten. Tarps used to collect the two guano samples from wild *T. brasiliensis* roosts were left undisturbed for six consecutive days

to allow for sufficient guano deposition. Guano was removed from tarps using a spatula and analyzed in the Departments of Zoology and Geological Sciences at the University of Florida. Care was taken to avoid contaminating guano with urine during the guano collection process.

All guano samples were homogenized in deionized water using a Philips 4-speed hand blender (model HR1358) and dried at 60°C for five days. Each sample was analyzed for dry matter (DM), organic matter (OM), lipid, carbon, nitrogen, and phosphorus. Sample analyses described below were performed in triplicate for each guano sample except for samples with insufficient mass.

To evaluate DM and OM, subsamples of homogenized guano were dried at 105°C for 16 hours, weighed, combusted at 500°C for three hours, and re-weighed. To evaluate lipids, subsamples of guano were weighed into cellulose thimbles. Lipids were extracted in a Dionex ASE300 Accelerated Solvent Extractor using 100% petroleum ether, and the resulting lipid-free sample was weighed. Subsamples of guano were analyzed for C and N content using a Carlo Erba NA 1500 CNS Elemental Analyzer. Guano was flash-combusted at 1,040°C, and the resulting N_2 and CO_2 gases were separated and passed through a thermal conductivity detector. Subsamples of guano were analyzed for P using a Bran-Lebbe Autoanalyzer with electronic data acquisition.

Nutrient composition was estimated on a percent dry matter (%DM) basis and percent organic matter (%OM) basis. Data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) prior to parametric analysis. Data for N and P content required a reciprocal transformation to improve the equality of variances. Data for C content (%OM) were not normally distributed (even when transformed with square root, square, natural log, or reciprocal transformations) and were therefore analyzed using a Kruskal-Wallis test, and pairwise differences in rank sums were evaluated using selected comparisons tests according to Langley (1971). Nutrient mass ratios (C:N, N:P, and C:P) were calculated from values expressed as %DM. Ratios of N:P required a square root transformation to improve equality of variances. Data, except C content (%OM), were tested for statistical significance using analysis of variance (ANOVA). When ANOVA revealed statistically significant differences among species, homogeneous subsets were determined using Student-Newman-Keuls (SNK) post hoc tests. Data were analyzed using SPSS for Windows (Release 11.0.0). For all reported analyses, values were significantly different if $P < 0.05$.

RESULTS

Most, but not all, of the response variables we measured were significantly different among species (Table 1). The OM (ANOVA, $F_{2, 6} = 0.69$, $P = 0.54$), lipid ($F_{2, 6} = 0.14$, $P = 0.87$ and $F_{2, 6} = 0.30$, $P = 0.75$ for %DM and %OM, respectively), and carbon (%DM) ($F_{2, 6} = 4.12$, $P = 0.08$) of guano did not differ significantly among the three bat species, although the difference in C (%DM) between *P. rodricensis* and *D. rotundus* guano approached significance (SNK, $n = 9$, $P = 0.07$). However, guano C (%OM) differed significantly (Kruskal-Wallis test, $\chi^2 = 6.49$, $d.f. = 2$, $P < 0.05$), with *D. rotundus* guano containing more C per unit of OM than guano of *P. rodricensis* (selected comparisons test, $P < 0.05$). Guano N differed significantly among the three species (ANOVA, $F_{2, 6} = 108.63$, $P < 0.001$ and $F_{2, 6} = 201.87$, $P < 0.001$ for %DM and %OM, respectively), with *D. rotundus* and *T. brasiliensis* guano containing more N than *P. rodricensis* guano (SNK, $n = 9$, $P < 0.05$). Guano P also differed (ANOVA, $F_{2, 6} = 10.55$, $P < 0.05$), with *P. rodricensis* and *T. brasiliensis* guano containing more P than *D. rotundus* guano (SNK, $n = 9$, $P < 0.05$).

There were significant differences in guano mass ratios among species (ANOVA, $F_{2, 6} = 90.98$, $P < 0.001$ for C:N; $F_{2, 6} = 84.68$, $P < 0.001$ for N:P; and $F_{2, 6} = 10.74$, $P < 0.05$ for C:P — Table 2). *Pteropus rodricensis* guano had a higher C:N ratio than guano of either *D. rotundus* or *T. brasiliensis* (SNK, $n = 9$, $P < 0.05$). The higher C:N ratio in *P. rodricensis* guano is a result of significantly lower N rather than higher C relative to *D. rotundus* and *T. brasiliensis* guano. The N:P and C:P ratios also differed significantly among species, with *D. rotundus* guano having higher N:P and C:P ratios than guano of both *P. rodricensis* and *T. brasiliensis* (SNK, $n = 9$, $P < 0.05$). The high N:P and C:P ratios in *D. rotundus*

TABLE 1. Composition of guano ($\bar{x}$ and SD) from three bat species ($n = 3$ for each species) as proportions of dry matter (DM) and organic matter (OM) content. Different superscripts indicate statistically significant differences ($P < 0.05$) between species within columns. Data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) prior to parametric analysis and reciprocal-transformed, if necessary. Data for C content (%OM) were not normally distributed (even when transformed) and were therefore analyzed using a Kruskal-Wallis test followed by a selected comparisons test. Data, except C content (%OM), were tested for statistical significance using analysis of variance with Student-Newman-Keuls post hoc tests

Organic matter		Lipid				C				N				P	
%DM		%DM		%OM		%DM		%OM		%DM		%OM		‰DM	
$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Pteropus rodricensis															
88.80	5.07	9.58	4.69	10.93	5.68	40.50	6.53	45.42[a]	4.90	1.95[a]	0.23	2.19[a]	0.17	5.71[a]	7.00
Desmodus rotundus															
88.36	1.53	17.74	4.30	20.08	4.78	50.52	2.79	57.19[b]	3.47	17.38[b]	9.56	19.62[b]	10.62	0.73[b]	0.25
Tadarida brasiliensis															
84.32	7.19	20.00	6.03	24.22	9.52	44.53	2.26	52.93[ab]	2.22	8.48[b]	1.78	9.98[b]	1.33	8.30[a]	4.49

DISCUSSION

Differences in guano composition (C, N, P, and mass ratios) most likely resulted from dissimilarities in nutrient composition of the diets of each bat species (Studier *et al.*, 1994). In addition, variation in physiological processing and assimilation of diets among the three species may have contributed to differences in guano nutrient composition. The differences in elemental composition and stoichiometric relationships we observed coupled with differences in resource needs among organisms living on or in bat feces may lead to differences in community structure among guano-based ecosystems.

The significantly higher carbon content in *D. rotundus* guano compared to that of *P. rodricensis* guano may be due to the fact that carbon contained in indigestible components of the fruits consumed by *P. rodricensis* is often excluded from feces. Although fruit bats consume a diet containing relatively high concentrations of structural carbohydrates (e.g., cellulose and hemicellulose), they differentially ingest juices and soft fruit pulp by straining out the more fibrous components as ejecta or pellets (Kunz and Diaz, 1995; Entwistle and Corp, 1997; Wendeln *et al.*, 2000). Ejecta are typically discarded prior to defecation, as production of ejecta requires approximately 2–4 min (Nyhagen *et al.*, 2005) while food transit time through the digestive tract averages 15–35 min (Morrison, 1980). For this reason, we did not include ejecta in our analyses of guano composition. The non-significant trend for C of *T. brasiliensis* guano to be greater than that of *P. rodricensis* guano

guano resulted from higher N, higher C, P, and mass ratios) most likely resulted and lower P in guano of *D. rotundus* compared to guano of *P. rodricensis* and *T. brasiliensis*.

may result from the presence of structural carbohydrates such as chitin in an insectivorous diet (and therefore in the feces). Although chitinase of bacterial origin has been detected in insectivorous bats, these enzymes do not apparently contribute substantially to digestion of heavy chitinous insect parts by these bats (Whitaker *et al.*, 2004). Unfortunately, we were unable to confirm differences in chitin content among samples because the mass of the *T. brasiliensis* guano samples was insufficient.

Our results for N agree with those of Studier *et al.* (1994), who found higher N in feces of insectivorous or omnivorous versus herbivorous bats. The differences among the species we tested likely resulted from higher protein concentrations in animal than in plant tissues. Despite the potential for nutrient dilution resulting from higher quantities of undigested structural components in insectivorous versus sanguivorous bat feces, chitin is a nitrogenous polysaccharide. Therefore, the fact that *D. rotundus* guano and *T. brasiliensis* guano were comparable in nitrogen could be explained by

the presence of undigested chitin in *T. brasiliensis* feces.

In contrast to our results for P, Shahack-Gross *et al.* (2004) found substantially greater phosphate concentrations in guano of wild insectivorous bats than in guano of wild frugivorous bats. We therefore expected P to be higher in *T. brasiliensis* guano than in *P. rodricensis* guano. The lack of a statistically significant difference between *T. brasiliensis* and *P. rodricensis* may partly result from very large variation in P within these two species. Goveas *et al.* (2006) similarly reported high levels of P in guano of frugivorous bats (*Pteropus giganteus*). We also expected frugivorous bat guano to contain lower lipid than insectivorous or sanguivorous bat guano. However, our study indicated that guano lipid was not significantly different among the species tested.

Variation in nutrients and stoichiometric nutrient ratios of guano from bats in different feeding guilds could have considerable effects on producers, consumers, and decomposers living on or in guano. As highlighted by Sterner and Elser (2002) and subsequently in reviews by Vrede *et al.* (2004) and Moe *et al.* (2005), relationships among elemental nutrients have the potential to regulate processes at many ecological levels, including production, individual and population growth, coexistence of species, rates of decomposition of organic matter, and nutrient cycling.

Primary production in terrestrial ecosystems (as in marine systems) is thought to be limited by the availability of N and P (Vitousek and Howarth, 1991), and the input of these nutrients by fecal deposition can have considerable bottom-up influences in detritus-based ecosystems. Nitrogen and/or phosphorous can also limit bacterial production and growth of consumers (Vrede *et al.*, 2004). We therefore suggest that our observed differences in nutrients in

TABLE 2. Nutrient mass ratios of guano from each of three bat species (*n* = 3 for each species). Values for ratios ($\bar{x}$ and SD) represent the quotient of the average concentration of one nutrient (% dry matter) and the average concentration of another nutrient (% dry matter) for each guano sample. Different superscripts indicate statistically significant differences ($P < 0.05$) between species within columns according to analysis of variance with Student-Newman-Keuls post hoc tests. Data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) prior to parametric analysis and square root-transformed, if necessary

C:N		N:P		C:P	
$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Pteropus rodricensis					
20.8[a]	2.3	9.4[a]	8.0	207.9[a]	188.3
Desmodus rotundus					
3.5[b]	1.6	227.6[b]	47.6	749.4[b]	270.7
Tadarida brasiliensis					
5.4[b]	1.0	11.5[a]	3.6	63.6[a]	28.2

guano of bats with different feeding habits could have substantial effects on primary and secondary productivity in guano-based ecosystems. These effects could result from differences in decomposition and nutrient cycling within guano deposits. The suites of decomposers and detritivores present in decaying organic matter appear to be determined by stoichiometric relationships among nutrients (Hessen *et al.*, 2004). Although both bacteria and fungi contribute to organic matter decomposition, these two groups are stoichiometrically disparate; fungi tend to have higher C:N and C:P ratios than bacteria. Furthermore, bacteria and fungi have different patterns of homeostatic regulation of cellular stoichiometries depending on the nutrient content of the medium on which they grow (Sterner and Elser, 2002). Substrates with dissimilar elemental stoichiometries may therefore harbor different assemblages of decomposers. Ecosystem-level effects of different nutrient contents could also result from differences in rates of conversion of nutrients in guano from biologically unavailable to available forms (Vitousek *et al.*, 1988).

Because guano from *P. rodricensis* and *D. rotundus* was collected from captive animals, we acknowledge the inherent limitations on extrapolating our findings to natural systems. For example, different geographic locations have different foods available for consumption by bats. Even wild bats in different roosts within the same population can consume different suites of diet items (Entwistle and Corp, 1997). However, the captive bats from which we collected guano consumed natural food sources (fruits and bovine blood, respectively) rather than synthetic diets, so their guano should nonetheless reflect approximate differences in composition. Despite this limitation, our study confirms that composition of bat guano varies with the dietary strategy of the bat. This variation includes differences

in concentrations of elemental nutrients as well as dissimilarities in stoichiometric relationships among nutrients. Differences in guano nutrient profiles could have considerable ecological consequences ranging from effects on the growth or productivity of individual residents of guano piles to effects on ecosystem-level processes like decomposition and nutrient cycling.

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