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SOIL MICROBIOLOGY

Desert Gerbils Affect Bacterial Composition of Soil

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Abstract Rodents affect soil microbial communities by burrow architecture, diet composition, and foraging behavior. We examined the effect of desert rodents on nitrogen-fixing bacteria (NFB) communities by identifying bacteria colony-forming units (CFU) and measuring nitrogen fixation rates (ARA), denitrification (DA), and CO_2 emission in soil from burrows of three gerbil species differing in diets. *Psammomys obesus* is folivorous, *Meriones crassus* is omnivorous, consuming green vegetation and seeds, and *Dipodillus dasyurus* is predominantly granivorous. We also identified NFB in the digestive tract of each rodent species and in *Atriplex halimus* and *Anabasis articulata*, dominant plants at the study site. ARA rates of soil from burrows of the rodent species were similar, and substantially lower than control soil, but rates of DA and CO_2 emission differed significantly among burrows. Highest rates of DA and CO_2 emission were measured in *D. dasyurus* burrows and lowest in *P. obesus*. CFU differed among bacteria isolates, which reflected dietary selection. Strains of cellulolytic representatives of the family Myxococcaceae and the genus *Cytophaga* dominated burrows of *P. obesus*, while enteric Bacteroides dominated burrows of *D. dasyurus.* Burrows of *M. crassus* contained both cellulolytic and enteric bacteria. Using discriminant function analysis, differences were revealed among burrow soils of all rodent species and control soil, and the two axes accounted for 91 % of the variance in bacterial occurrences. Differences in digestive tract bacterial occurrences were found among these rodent species. Bacterial colonies in *P. obesus* and *M. crassus* burrows were related to bacteria of *A. articulata*, the main plant consumed by both species. In contrast, bacteria colonies in the burrow soil of *D. dasyurus* were related to bacteria in its digestive tract. We concluded that gerbils play an important role as ecosystem engineers within their burrow environment and affect the microbial complex of the nitrogen-fixing organisms in soils.

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Introduction

A variety of animal taxa serve as ecosystem engineers in different biotas. Their impacts differ, and their effects could be direct or indirect [52]. One of the main ways in which rodents affect the surrounding environment is through their burrowing activity. Rodent burrows provide habitats for many vertebrate and invertebrate animals [12, 19, 55, 82, 100] and a suitable environment for the estivation and early development of seeds [13]. In addition, burrowing affects the aeration and water regime of the soil, its structure, and microtopography [46, 49, 74].

Most studies on the effect of rodent burrowing activity on soils have focused on the chemical and physical properties of the soil [13, 64, 71, 91, 93] and/or on the change in vegetation associated with the burrows [11, 16, 38, 45, 78, 99]. The

effect of rodent burrows on biological properties of soils and soil microbial community has received less attention [1, 6, 18, 22, 34, 43, 44, 60]. Results of the studies on the effects of rodent burrowing activity have been equivocal. For example, it was reported that urine and feces stimulate microbial activity [43, 67, 68]. However, some animals reduce the average soil nitrogen content by moving nitrogen-poor subsurface soil to the soil surface [33, 48, 83] and, thus, depress microbial activity.

Rodents may affect soil microbial communities by their burrow architecture, diet composition, and foraging behavior. Consequently, burrows belonging to different rodent species may present different environments to soil microbial communities. These effects are expected to be most pronounced in soils of arid lands, where organic matter and nitrogen contents are typically low [7, 30, 70].

We studied the composition of nitrogen-fixing bacterial communities in the burrows of three coexisting rodent species in loess soils in the central Negev Desert and measured rates of nitrogen fixation, denitrification, and CO_2 emission in these soils. The three rodent species differ in their dietary habits (granivorous, folivorous, and omnivorous—both seeds and green vegetation) and foraging behavior. We predicted that soil from burrows would be characterized by (a) higher microbial abundance and activity but (b) lower rates of nitrogen fixation compared to soils from the same depth at sites not inhabited by rodents. The basis for this prediction is that burrowing animals disrupt the soil crust in which nitrogen-fixing microorganisms congregate. We also predicted that soil from burrows would have higher rates of CO_2 emission because of accumulation of organic matter (e.g., feces, food plant remnants, etc.). In addition, we expected that dietary habits of rodents would affect relative abundances of cellulolytic bacteria in soils of their burrows. We predicted that more cellulolytic bacteria with lower rates of N_2-fixing activity in the microbial community would occur in burrows of herbivorous rodents than granivorous rodents. To test whether the effect of rodents on microbial species composition in their burrows is mainly associated with either plant remnants or rodent feces, we compared microbial species composition in burrow soil with those in intact plants and in the digestive tracts of rodents. This is the first study that relates the bacterial community profile in soil from a rodent burrow to that of the digesta and dietary habits of the rodent.

Materials and Methods

Study Area

The study was carried out in the summer (June–July) at *wadi* Nizzana, an ephemeral river, at the northern rim of the Ramon erosion cirque, Negev Highlands, Israel (30°35′N, 34°41′E; 790 m a.s.l.). This area consists of a complex of hills with a deep loess layer and wide dry riverbeds densely covered with shrubs of *Anabasis articulata* and *Atriplex halimus*. Summers are hot and dry (mean daily air temperature in July is 34 °C), and winters are relatively cool (mean daily temperature in January is 12.5 °C). Mean annual rainfall in the area is approximately 80 mm, all falling in the winter [80]. The study site (100×50 m) included the first fluvial terrace of the Nizzana riverbed.

Rodent Species

We compared biological properties of the soil taken from burrows of three coexisting gerbilline species that differ in body mass and dietary habits. *Psammomys obesus* (Cretzschmar, 1828) is a large (130–200 g), strictly folivorous, diurnal species that feeds mainly on chenopods such as *A. articulata* and *A. halimus* [14, 17, 53], *Meriones crassus* (Sundevall, 1842) is a medium-sized (50–110 g), omnivorous, nocturnal gerbil that feeds on seeds and green vegetation [54, 58], and *Dipodillus dasyurus* (Wagner, 1939) is a small (25–30 g), nocturnal gerbil that is predominantly granivorous [2, 37, 41, 94]. These rodents are protected in Israel, as are all wild animals, but are not considered endangered species. Burrows belonging to the three different rodent species can be distinguished easily by the size and shape of their burrow openings [28, 79]. We took soil samples from occupied burrows of each rodent species; occupation was verified by field observations. These are solitary rodent species [16, 41, 67, 92, 98] and, consequently, there was one individual in each burrow. *A. articulata* and *A. halimus* were the main shrubs available at the time of the study.

Digestive Tract Samples

During a long-term study of rodent species population dynamics, a number of individuals died accidentally, either in traps (due to the crushing of the traps by a large predator, most likely a wolf) or while handling. These rodents presented an opportunity to test possible effects of bacteria from their digestive tracts on bacterial communities in the soil. Five *P. obesus* and four individuals of each of the other two species that we knew were collected within 15 min of their death were used for identification of microorganisms in the digestive tract. The entire digestive tract of each animal was removed, and the connective tissues and lipids were carefully stripped. Samples were weighed to ±0.1 mg (Mettler balance, Toledo AB54), and the contents of the colon, cecum, and forestomach were used for bacterial cultivation. Symbiotic fermentation occurs in the forestomach and cecum, and the function of the colon is to separate the bacterial mass from the gut content and return it to the

cecum. Bacteria cultivations of the digestive tracts of each rodent specimen for each species were replicated four times.

Soil Samples

Soil samples of approximately 80 g each from 6 to 11 burrows of each species were collected from the burrow tunnel close to the entrance, at a depth of 15–30 cm. This site, close to the soil crust, was chosen because the highest concentration of nitrogen-fixing bacteria is expected there [4, 5, 96]. Nine soil samples from the same depth of uninhabited soil were used as a control. Preparation of soil samples followed standard methods [98]. In short, three to four samples from each burrow core were pooled, air-dried, and stored at room temperature until analyzed for nitrogen content, soil respiration, denitrification, nitrogen-fixing activity, and bacterial cultivation. Before analyses, 5 g of soil samples was moistened with 1 ml of water to field capacity (60 % of water holding capacity) and pre-incubated for 5 days at 20 °C to avoid a flush of microbial activity [72, 81, 98]. Analyses of each pooled soil sample were replicated three times.

Plant Samples

The most common plant species in the study area were *A. articulata* and *A. halimus.* About 100 g of fresh green material from four *A. articulata* and five A. *halimus* plants was collected for nitrogen content analysis and bacterial cultivation. Before bacterial cultivation, a 10-g sample of each was washed three times in 200 ml of sterilized water to remove soil [9, 10]. Analyses of each plant sample were replicated four times.

Soil Nitrogen Content, Soil Respiration, and Denitrification Activity

Total nitrogen content of soil samples was determined using micro-Kjeldahl digestion [51] and a CHNS analyzer (VarioEL III, Elementar Analysensysteme GmgH, Hanau, Germany). Nitrogen content was determined by titration of ammonia with a standard solution of 0.1 H HCl in the presence of a mixed indicator (bromocresol green and methyl red) [51].

Soil respiration was measured by gas chromatography as the rate of CO_2 produced (CO_2 emission) from a 2-g sample during 96 h incubation at 28 °C (Chromatograph M 3700/4 JSC "Chromatograph," Moscow, Russia, with katharometer, length of the column 3 m, filler material Polysorb, transmitter gas He) [32].

Rate of denitrification activity was determined by gas chromatography as N_2O produced during incubation at 28 °C for 24 h (Chromatograph M 3700/4; see details above). Five grams of pre-incubated soil was placed in a penicillin flask and then 2.5 mg glucose, 1.5 mg KNO_3, and 5 ml of water were added. The flasks were closed with rubber plugs, the air inside the flask was replaced with argon (argon at a high-pressure was forced through the flask during 40 s, air volume was displaced by the vent) to produce anaerobic conditions, and 1 ml acetylene was added [24, 35, 95].

Nitrogen-Fixing Activity

The potential rate of nitrogen fixation in soil was determined in aerobic conditions by the acetylene (C_2H_2) reduction assay (ARA) using gas chromatography [15, 21, 40]. In this method, acetylene is reduced to ethylene (C_2H_4) due to nitrogenase enzyme activity [21], and ethylene formation in nanomoles per gram soil per hour is calculated. Five grams of pre-incubated soil samples were placed in glass vials (15 ml volume), and 1 ml of 10 % glucose solution was added to 5 g of soil and incubated at 28 °C for 24 h to provide active growth of microorganisms. Then, the flasks were closed tightly with rubber plugs, and 1 ml of the gas phase in each flask was replaced by acetylene via a syringe. The flasks were incubated at 28 °C for a further 1 h, and a 1 ml sample of the gas phase was collected from each flask for chromatograph analysis (Chromatograph "Chrom-4" Laboratorni pristroje, Prague, Czech Republic, with a flame ionization detector; length of the column 3.2 m, filler material Spherosil, transmitter gas Ar).

To determine nitrogen fixation in pure cultures, we used cultivation of isolated bacteria in Ashby's Fluid medium in penicillin flasks at 28 °C for 5 days. Flasks were then tightly closed with rubber plugs; further preparations for the analyses were described above. Nitrogen fixation was calculated per volume of culture suspension in flask [3, 39, 47, 57, 63].

Bacterial Cultivation and Identification

Ten grams each of soil, washed plant material of *A. articulata* and *A. halimus*, and digestive tract content were ground and shaken in a flask with distilled water for 3 min. A set of standard dilutions between 10^2 and 10^5 was used for bacterial cultivation. An aliquot of 40–50 μl for each dilution was plated on Ashby's Glucose-Agar medium surface (Sigma-Aldrich Corporation, St. Louis, MO, USA). The plates were incubated at 28 °C (FOC225E, Velp Scientifica srl, Milano, Italy) for 3–5 days, and the number of colony-forming units (CFU) per gram of sample was determined and log-transformed. The ability of identified microorganisms to fix N_2 was checked by ARA (for details see "Nitrogen-Fixing Activity" above). The isolated colonies were identified using conventional microbiological procedures based on phenotypic criteria [47, 63], including colony morphology, pigmentation, motility, oxidase reaction, and catalase coupled with Gram strain to a genus or family level. Possibility of aerobical or facultative anaerobical growth was determined while

cultivation on OF basal Hugh-Leifson medium (Merck Chemicals Inc. Gibbstown, NJ, USA) for microbiology.

Data Analyses

First, we tested whether total nitrogen content and rates of CO_2 emission, denitrification activity, and nitrogen-fixation activity (dependent variables) differed (a) between control and burrow soils and (b) among burrow soils from different rodents species. Prior to analyses, independent variables were either log or angular transformed. The transformations resulted in distributions that did not deviate significantly from normality for all dependent variables except for total nitrogen content. Therefore, non-parametric tests were used to analyze the latter variable (Mann–Whitney *U* test for two-group comparisons and/or Kruskal–Wallis ANOVA for multiple comparisons). To analyze the remaining dependent variables in triplicates of pooled soil samples, we used repeated measures ANOVA and factorial ANOVA for comparison among species and between soil from rodent burrows and control soils. Values are presented as means±SE.

We used stepwise discriminant function analysis (DFA) for the bacterial species composition and their relative abundances among soils, plants, and digestive systems of rodents. Before analyses, we calculated and angular-transformed proportion of each bacterial species within each sample. Confidence ellipses for the occurrences of bacterial communities in ordination space were calculated based on the assumption of a bivariate normal distribution. DFA was applied for ordination of bacterial communities in (1) digestive tracts among rodent species; (2) burrow soils among rodent species; and (3) digestive system, burrow soil, and plants within each rodent species. Differences were determined as significant Mahalanobis distances between group centroids.

Results

Soil Characteristics

Nitrogen contents in burrow soils of *D. dasyurus* (0.071 %; Mann–Whitney *U* test $Z=2.93$, $P=0.003$) and *M. crassus* (0.081 %; Mann–Whitney *U* test $Z=-2.611$, $P=0.009$) were higher than in *P. obesus* burrow soil (0.048 %), which was similar to control soil (0.054 %; Table 1).

Among burrows of rodent species, rates of ARA were similar ($df=2$; $F=1.74$; $P=0.24$) and averaged 2.51±0.43 nmole $C_2H_4 \cdot g^{-1}\ h^{-1}$, approximately 50 times lower ($df=3$; $F=22.78$; $P<0.001$) than that of control soil (114.52 nmole $C_2H_4 \cdot G^{-1}\ h^{-1}$; Table 1).

Denitrification activity (DA) rate in *D. dasyurus* burrows (2.74 nmole $N_2O \cdot g^{-1}\ h^{-1}$) was higher than in *P. obesus* burrows (1.43 nmole $N_2O \cdot g^{-1}\ h^{-1}$), and both differed from control soil ($F=10.17$; $P<0.001$ and $F=12.85$; $P=0.023$, respectively). DA rate in *M. crassus* burrows was intermediate (1.78 nmole $N_2O \cdot g^{-1}\ h^{-1}$) and was similar to control soil ($F=1.63$; $P=0.27$; Table 1).

CO_2 emission rate in the soil of *D. dasyurus* burrows (16.28 μmole $CO_2\ g^{-1}\ h^{-1}$) was higher ($df=3$; $F=7.01$; $P=0.012$) than that of *P. obesus* (10.16 μmole $CO_2\ g^{-1}\ h^{-1}$) and *M. crassus* (13.37 μmole $CO_2\ g^{-1}\ h^{-1}$), which were similar and did not differ from control soil ($df=2$; $F=3.50$; $P=0.098$; Table 1).

Bacterial Isolates

Not all the isolates were able to fix nitrogen. The highest ARA levels were detected in *Bacillus* (up to 2.43 nmole $C_2H_4\ ml^{-1}\ h^{-1}$), lower levels were detected in Nocardiaceae, Enterobacteriaceae, *Arthrobacter*, and *Aquaspirillum* (range 0.3–0.7 nmole $C_2H_4\ ml^{-1}\ h^{-1}$), and insignificant levels were detected in Myxococcaceae, *Cytophaga*, and *Micrococcus*. Based on these results, the isolates were divided into "nitrogen-fixing bacteria" and "other bacteria."

Control soil was dominated by *Bacillus* and *Arthrobacter* with some occurrence of *Cytophaga* and Myxococcaceae; the latter two are considered cellulolytic. The fraction of cellulolytic microorganisms in *P. obesus* and *M. crassus* burrows was higher than in control soil, while that of *D. dasyurus* was similar to control soil. Strains of *Aquaspirillum* were isolated from burrows of all rodent species but not from control soil. The enteric microorganisms Bacteroides were recorded only in *D. dasyurus* and *M. crassus* burrows (Table 2). Residual

Table 1 Total nitrogen content and rate of nitrogen-fixing activity (ARA), denitrification activity (DA), and CO_2 emission of soil samples

Variable	Control	*P. obesus*	*M. crassus*	*D. dasyurus*
Sample size	9	7	11	6
Total nitrogen (% of dry matter)	0.054±0.002a	0.048±0.001a	0.081±0.002b	0.071±0.006b
ARA (nmole $C_2H_4\ g^{-1}\ h^{-1}$)	114.52±23.30b	2.09±0.54a	3.45±0.96a	1.85±0.32a
DA (nmole $N_2O\ g^{-1}\ h^{-1}$)	2.00±0.15b	1.43±0.13a	1.78±0.16ab	2.74±0.17c
CO_2 emission (μmole $CO_2\ g^{-1}\ h^{-1}$)	12.70±0.50a	10.16±1.58a	13.37±0.77a	16.28±1.18b

Values are presented as means±SE. Within rows, different lowercase letters are significantly different from each other ($P<0.05$)

Table 2 Bacterial counts (log colony-forming units per gram dry matter; means±SE) on nitrogen-free medium in soils from rodent burrows and from plants

	Soil cultures					Plants		
Sample size	Control 9	*P. obesus* burrow 7	*M. crassus* burrow 11	*D. dasyurus* burrow 6	p	*Atriplex halimus* 5	*Anabasis articulata* 4	*P*
Nitrogen-fixing bacteria								
Bacillus spp.	5.70±0.23a	5.52±0.11a	5.39±0.22a	2.87±1.29b	<0.01	3.36±0.13	3.73±0.30	ns
Arthrobacter	5.88±0.16a	1.64±1.06b	3.55±0.70b	2.86±1.28b	<0.01	1.64±1.01	2.96±0.13	ns
Nocardiaceae	0.60±0.60	0.00	0.00	0.00	ns	0.00	0.00	–
Aquaspirillum	0.00a	1.66±1.07ab	1.66±0.71ab	2.22±0.99b	<0.05	0.92±0.57	0.00	–
Bacteroides	Noa	0.00a	2.73±0.79b	3.78±1.20b	<0.01	0.00	0.00	–
Staphylococcus	1.21±0.80	0.00	0.00	0.00	–	0.00	0.00	–
Other bacteria								
Myxococcaceae[a]	0.62±0.62a	3.15±1.11bc	3.11±0.76b	0.73±0.73ac	<0.05	1.44±0.88	1.90±1.12	ns
Cytophaga[a]	1.64±0.85ab	3.60±0.96a	1.90±0.80ab	0.00b	ns	1.91±0.79	1.67±0.99	ns
Micrococcus	0.00	0.00	0.00	0.00	–	1.47±0.90	0.00	–

Within rows, different lowercase letters are significantly different from each other ($P<0.05$)

[a] Microorganisms associated with plant substrates

levels of Nocardiaceae and *Staphylococcus* were detected in control soil but not in the rodent burrows.

The CFU of the various bacteria isolates in the digestive tract differed significantly among rodent species. *Cytophaga* and *Arthrobacter* were absent in *M. crassus*, while *Staphylococcus*, *Micrococcus*, and Enterobacteriaceae were absent in *P. obesus* (Table 3).

Levels of the different bacteria in the soil, in the halophytic plants, and in the digestive tract of the rodents were highly variable (Tables 2 and 3); however, significant differences emerged in bacteria levels among samples, suggesting differences in bacteria composition among rodent burrows.

Summary of DFAs is presented in Table 1S. Despite broad overlap of soil bacterial communities among rodent burrows and control soil, differences among all were significant. *P. obesus* and *M. crassus* were close to each other, and both were distant from *D. dasyurus* (Table 2S, Fig. 1a). The first two axes accounted for 96 % of the variance in bacterial occurrences (Table 1S). Differences in soil characteristics were mostly influenced by three bacterial isolates, namely, *Bacillus*, Myxococcaceae, and Enterobacteriaceae (Table 1S).

DFA showed significant differences in digestive tract bacteria composition among the three rodent species; the first two axes accounted for 100 % of the variance in bacterial

Table 3 Bacterial counts (log colony-forming units per gram dry matter; means±SE) on nitrogen-free medium in digestive tracts of rodents

Sample size	*P. obesus* 5	*M. crassus* 4	*D. dasyurus* 4	*P*
Nitrogen-fixing bacteria				
Bacillus	2.10±0.72a	4.90±0.83b	4.97±1.04b	<0.05
Arthrobacter	2.44±0.54b	0.00a	3.41±1.02b	<0.01
Nocardiaceae	0.97±0.49	1.24±0.69	2.55±1.08	ns
Aquaspirillum	0.78±0.37a	4.72±0.83b	2.59±1.11ab	<0.01
Bacteroides	2.65±0.63b	0.78±0.54a	6.20±0.78c	<0.01
Staphylococcus	0.00a	1.51±0.80ab	2.28±0.99b	<0.05
Other bacteria				
Myxococcaceae[a]	1.15±0.47a	3.44±0.97b	5.28±1.06b	<0.01
Cytophaga[a]	3.35±0.62b	0.00a	5.21±1.05c	<0.01
Micrococcus	0.00a	1.80±0.82b	1.12±0.76ab	<0.05
Enterobacteriaceae	0.00	1.02±0.73	0.72±0.72	ns

Within rows, different lowercase letters are significantly different from each other ($P<0.05$)

[a] Microorganisms associated with plant substrates

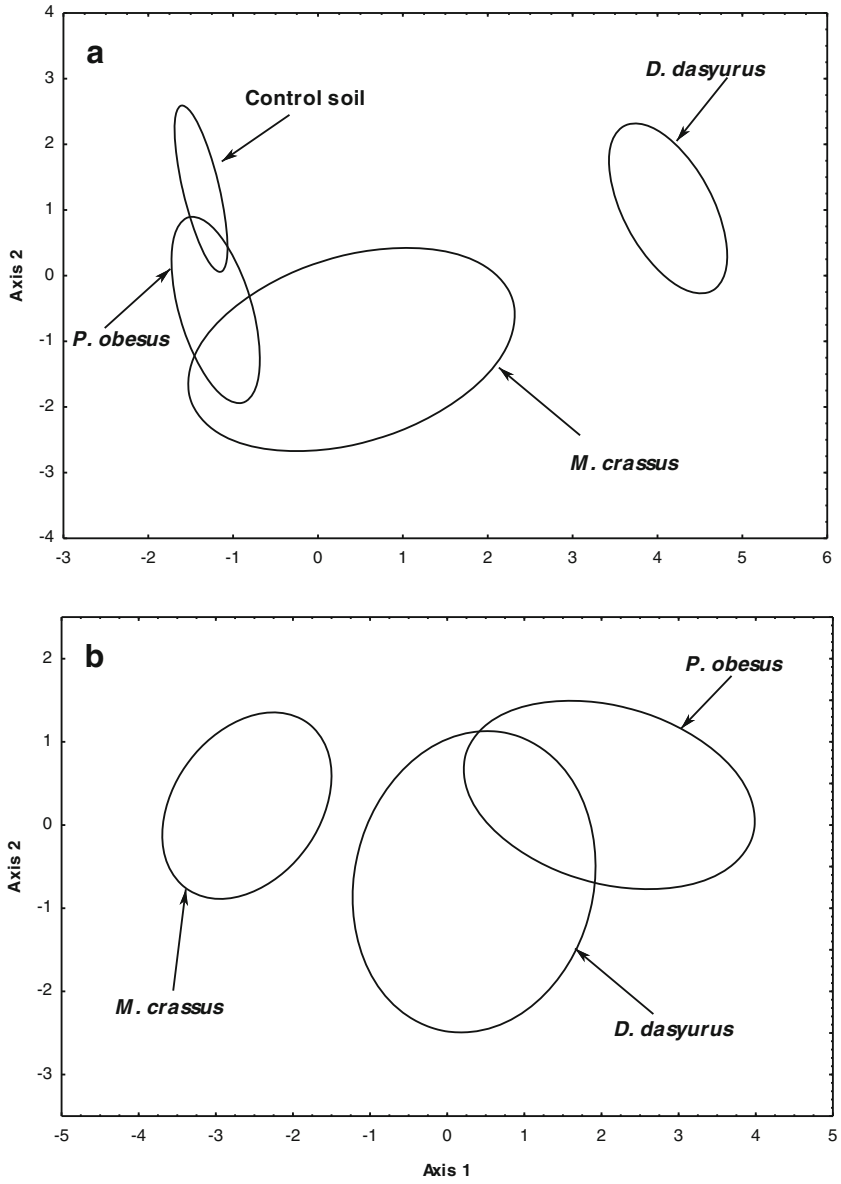

Fig. 1 Discriminant function analysis for nitrogen-fixing bacterial communities in the burrow soil (**a**) and in the digestive tract (**b**) of *P. obesus*, *M. crassus*, and *D. dasyurus* (see detailed statistics and Mahalanobis distances in Tables 1S, 2S, and 3S)

occurrences (Tables 1S and 3S, Fig. 1b). Differences in digestive tract bacterial communities were influenced mostly by five bacterial isolates, namely, *Cytophaga*, *Arthrobacter*, *Bacillus*, Nocardiaceae, and Enterobacteriaceae (Table 1S).

DFA revealed significant differences among bacteria communities among plants, burrow soil, and digestive tract within each rodent species. Bacterial composition of burrow soils was significantly different from that of control soil for all rodents; DFA for both *P. obesus* and *M. crassus* indicated similarity between bacterial composition in burrow soil and that of the plant *A. articulata*; differences between burrow soil and either *A. halimus* or the digestive tract were significant (Table 3S, Fig. 2a, b). However, a different pattern was found for *D. dasyurus* where bacteria composition in burrow soil was similar to that in the digestive tract; differences between burrow soil and both plants were significant (Table 3S, Fig. 2c). The two axes accounted for 67, 87, and 91 % of the variance in bacterial occurrences for *P. obesus*, *M. crassus*, and *D. dasyurus*, respectively (Table 1S). Differences in bacterial communities were mostly influenced by two bacterial isolates, Enterobacteriaceae and *Micrococcus*, in *P. obesus*, by five bacterial isolates, *Arthrobacter*, *Cytophaga*, Enterobacteriaceae, Myxococcaceae, and *Bacillus*, in *M. crassus*, and by four bacterial isolates, Enterobacteriaceae, *Arthrobacter*, *Bacillus*, and *Aquaspirillum*, in *D. dasyurus* (Table 1S).

Discussion

Testing of the N_2-fixing ability of the identified representatives of Enterobacteriaceae and Nocardiaceae, and the genera *Bacillus*, *Aquaspirillum*, and *Arthrobacter* showed that all are

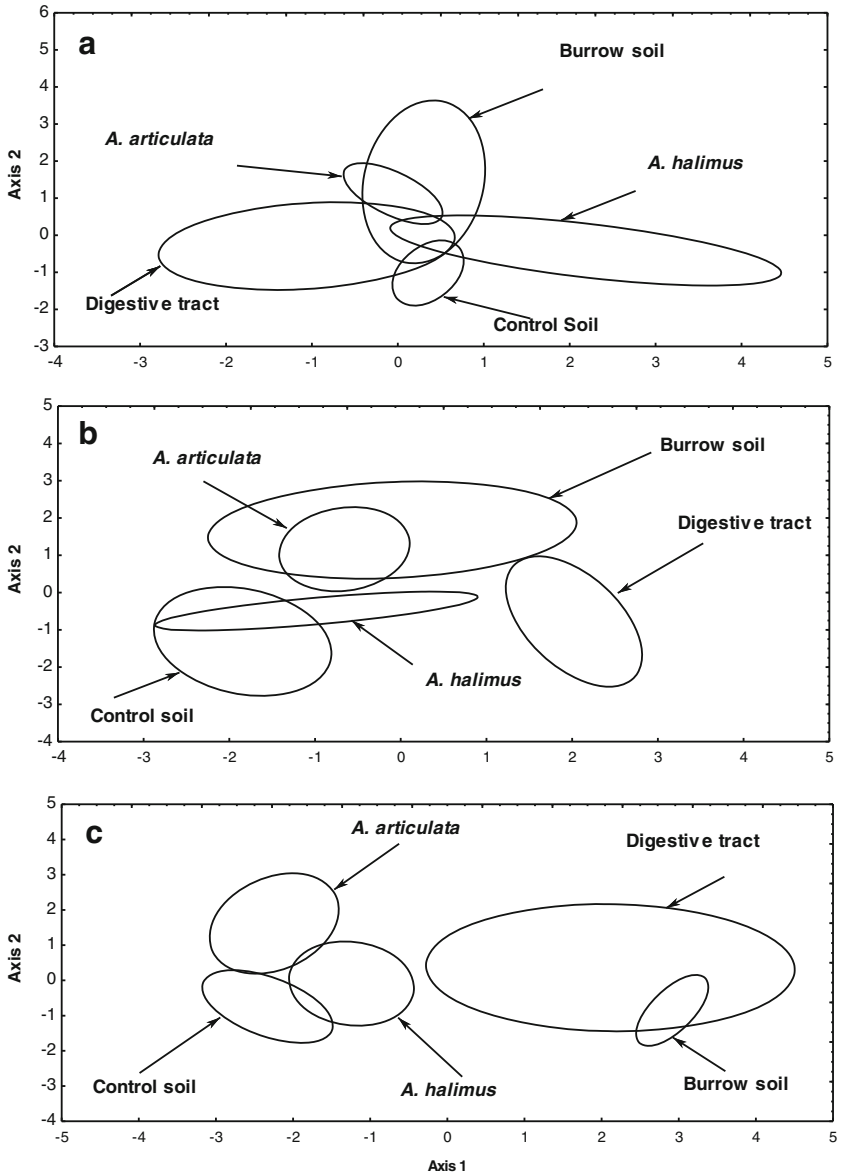

Fig. 2 Discriminant function analysis within each rodent species for nitrogen-fixing bacterial communities in *A. halimus*, *A. articulate*, and in the burrow soil and digestive tract of **a** *P. obesus*, **b** *M. crassus*, and **c** *D. dasyurus* (see detailed statistics and Mahalanobis distances in Tables 1S and 4S)

nitrogen-fixing, as was reported by other investigators [8, 25, 73, 84, 87, 97]. The isolation of non-N_2-fixing bacteria on the N-free media was predictable. There are two principle reasons for their presence: (1) some microorganisms appear to meet their nitrogen requirements by scavenging atmospheric ammonia [73]; and (2) close proximity between nitrogen and non-nitogen fixing bacteria, for example, where non-diazotrophic cellulolytic bacteria provide metabolites as a carbon source for diazotrophs and obtain fixed nitrogen [50, 73, 76]. N_2-fixing bacteria in these shrubs have not been reported previously. In addition, the occurrence of N_2-fixing bacteria in the digestive system of these rodents is of interest as ammonia levels are sufficiently high to make N_2 fixation a costly and unnecessary metabolic activity. However, the presence of N_2-fixing bacteria in the gut has been reported for some rodent species [65, 69, 88], and the importance of N_2-fixing bacteria in the digestive system of mammalian species consuming low nitrogen diets was discussed [29, 31, 59, 66, 89].

Our main hypothesis was supported in that bacterial communities differed among rodent burrows as well as from control soil (Fig. 1a). Changes in soil bacterial communities due to mammalian activities have been reported in other studies [1, 20]. Factors determining bacterial communities in the burrows could be separated into three sources: (1) food caching, (2) excreta (urine and feces) of the inhabitant, and (3) burrowing activity [56, 61, 62].

All three rodent species collect food which they cache in their burrows [36, 42, 80, 86]. *D. dasyurus* caches only seeds, *P. obesus* only green vegetation, and *M. crassus* caches both seeds and green vegetation. In our study site, the main plant selected and, consequently, cached in burrows by both *P. obesus* and *M. crassus* was *A. articulata* [36, 85, 86]. Bacterial

communities in burrows of both of these rodent species were similar to that of *A. articulata*, which was not the case for *D. dasyurus*. We reasoned that the cellulolytic bacteria, *Cytophaga* and representatives of the family Myxococcaceae, were introduced into the burrow with the cached plants. Similarly, cellulolytic microorganisms were dominant in the soil from the paths and burrow walls of the common vole, a herbivorous rodent that consumes green vegetation [6].

DFA showed that the bacterial communities in the *D. dasyurus* burrows were closely related to the bacteria of the digestive tract. We reasoned that this was due to fecal remains in the burrow. The enteric bacteria, Enterobacteriaceae, were present in both *D. dasyurus* and *M. crassus* burrows indicating fecal remains in the burrow; fecal remains were reported for *M. crassus* [80]. Although Enterobacteriaceae was present, we could not find similarity in bacterial communities between the digestive tract and burrow soil of *M. crassus* due to, most likely, the larger effect of *A. articulata* on its bacterial community. No Enterobacteriaceae was detected in burrows of *P. obesus*. There are indications that burrows of *P. obesus* are feces-free, as this rodent defecates outside its burrow and the entrance to an active burrow is maintained clean of feces constantly [28, 85, 86]. While cellulolytic bacteria were also isolated in sporadic amounts (<5 %) from the burrows of the granivorous *D. dasyurus*, more enteric organisms were observed in their burrows. The fraction of the enteric group of microorganisms (Enterobacteriaceae) increased with the fraction of seeds in the diet from total absence in *P. obesus* burrows to 34 % in *D. dasyurus* burrows.

The overall high rate of nitrogen fixation in control soil was predicted and is well documented [7, 26, 77, 92, 96]. Decreased ARA rates in burrows can be explained by the disturbance of the soil by animals [27]. Furthermore, high nitrogen content suppresses nitrogenase activity [75]. Our results indicate that the three rodent species decreased nitrogen-fixing activity rates but differed in nitrogen accumulation in their burrows. Total nitrogen contents in burrows of *M. crassus* and *D. dasyurus* were almost twice as high as in control soil (Table 2). Urine and feces contribute to soil nitrogen content [90], and these rodents defecate in their burrows. Rates of CO_2 emission and DA indicate potential decomposition of organic matter. *P. obesus* do not defecate inside burrows and, hence, the low rates of these measurements. The relatively higher nitrogen and fecal remains in *D. dasyurus* burrows is in accordance with highest denitrification activity and CO_2 emission rates in the soil of their burrows.

In this preliminary study, bacterial communities were identified phenotypically and, for the first time, the interaction among bacterial communities in soil, plants, and animals was measured. In future studies, 16S ribosomal DNA-based identification of bacteria will also be used [23] to characterize these communities and present a better picture for the taxonomic changes due to rodent activity in the soil.

We concluded that gerbils play an important role as ecosystem engineers within their burrow environment and affect the microbial complex of the nitrogen-fixing organisms in soils. Furthermore, the responses are not uniform and stem from the diversity of dietary and behavioral habits employed by each gerbil species.

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Conflict of Interest The authors declare that they have no conflict of interests.

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