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Effects of temporally persistent ant nests on soil protozoan communities and the abundance of morphological types of amoeba

Salvador Rodriguez Zaragoza [a], *Walt G. Whitford* [b], *Yosef Steinberger* [c,*]

[a] *Lab. de Microbiología, Unidad de Biología Tecnología y Prototipos, Facultad de Estudios Superiores Iztacala, UNAM, Avenida de los Barrios #1, Los Reyes Iztacala, Tlalnepantla, Estado de México, México 54090, Mexico*

[b] *USDA-ARS Jornada Experimental Range, P.O. Box 30003, MSC 3JER, New Mexico State University, Las Cruces, NM 88003, USA*

[c] *The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel*

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ABSTRACT

We compared soil protozoan communities near ant nests with soil protozoans in reference soils 5 m from the edge of ant mounds. We sampled three species of Chihuahuan Desert ants that construct nests that persist for more than a decade: a seed harvester, *Pogonomyrmex rugosus*, a liquid feeding honey-pot ant, *Myrmecocystus depilis*, and a generalist forager, *Aphaenogaster cockerelli*. Ant colonies were located on different topographic positions on catenas of two watersheds. Total protozoan abundance was higher in *P. rugosus* nest soils at the top of a catena and in *A. cockerelli* nest soils in a grassland than in the respective reference soils. There were qualitative and quantitative differences in protozoan communities associated with the nests of ants at all locations studied. Amoebae were the most abundant protozoans at all locations. Type 1 amoebae (flattened with sub-pseudopodia (like *Acanthamoeba*)) occurred at the highest frequency and was the only amoeba type found in *M. depilis* nest soils and *P. rugosus* nest soils at the top of a catena. Nanoflagellates were associated with *P. rugosus* and *M. depilis* nest soils but were absent from reference soils. Ciliates, testate amoebae and nanoflagellates were absent from *A. cockerelli* reference soils but were present in nest soils. The effects of ants on soil protozoan communities depend on the temporal persistence of the colony, nest building and food handling behavior, topographic position and soil type.

1. Introduction

There are a number of studies at single locations and one ant species that report increased concentrations of nutrients and plant biomass around the nests of seed harvesting ants (MacMahon et al., 2000). A study of the effects of the seed harvesting ant, *Pogonomyrmex rugosus*, on soil nutrients on a Chihuahuan Desert watershed reported than soil nutrients were concentrated in nest associated soils in some but not all locations on the watershed (Whitford and DiMarco, 1995). That study emphasized the potential for differences in the effects of ant nests on soils related to landscape position, geomorphic surface and soil characteristics. Our studies focused on persistent ant nests on two catenas in the northern Chihuahuan Desert in order to evaluate spatial effects.

Two recent studies reported increased diversity and abundance of soil biota (bacteria, fungi, nematodes, protozoans and microarthropods) in soils associated with harvester

* *Corresponding author*. Tel.: +972 3 5318571; fax: +972 3 7384058.
E-mail address: steinby@mail.biu.ac.il (Y. Steinberger).

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ant nests (Wagner et al., 1997; Boulton et al., 2003). With one exception (Wagner, 1997), these studies have focused on large body-size seed harvesting ants because they produce large nests (>1 m in diameter) which are continuously occupied for several decades, and as central place foragers, accumulate organic matter in the vicinity of the nests (Wagner and Jones, 2004). Wagner (1997) reported that the nests of *Formica perpilosa*, a honey-dew feeder—predator/scavenger (Schumacher and Whitford, 1974) increased the concentration of soil nitrogen and phosphorous. The study of *F. perpilosa* suggests that central place foragers that build and occupy large subterranean nests for several decades may affect soil properties in ways similar to seed harvesting ants. In order to test this hypothesis, we designed a study to compare the effects of nests of a seed harvesting ant (*P. rugosus*) known to affect soil properties with the effects on soils of nests of two species of ants that are not seed harvesters, namely *Aphaenogaster* (*Novomessor*) *cockerell*, a collector of detritus, seeds and insects, and a honey-pot ant, *Myrmecocystus depilis*, a species that collects plant exudates, honey dew and small insects.

Although protozoans are an important component of the soil biota, there are few studies that report the abundance and composition of the protozoan community in arid region soils (Bamforth, 1984, 2004; Parker et al., 1984; Wagner et al., 1997). As one test of the hypotheses on the effect of persistent nests of central place foraging ants on soil biota, we initiated a detailed study of soil protozoans from soils associated with three species of Chihuahuan Desert ants at five locations on two different catenas. We hypothesized that the protozoan communities associated with persistent ant nest soils would differ significantly from the protozoan communities in reference soils.

2. Methods

Samples were collected at four sites on a watershed at the Chihuahuan Desert Rangeland Research Center (CDRRC) located 50 km NNW of Las Cruces, New Mexico, and at one site in the Nutt grasslands located approximately 30 km W of Hatch, NM. Soil cores (20 cm deep–10 cm diameter) were collected from five ant nest mounds or discs and five reference points located in a random direction, 5 m from the ant nest. Honey-pot ants, *M. depilis*, nests were located on an upper piedmont slope with coarse sandy soils with a sparse cover of creosotebush, *Larrea tridentata*. Nests of the seed harvester, *P. rugosus*, were sampled at the base of the watershed (basin) on a gently sloping catena (<2% slope) with three geomorphic surfaces and soil types. The upper site with sandy-loam soil was separated from the mid-level site by a 0.5 m escarpment. The dominant vegetation that characterized this site was an annual plant, *Cryptantha angustifolia*. The mid-slope site receives run-off water from the upper site and has a fine loam soil. The dominant vegetation that characterized this site was the spring annual plant, *Erodium texanum*. The lowest site on the catena is a run-on location with clay-loam soil which supported a mixture of several species of spring annuals. The perennial vegetation on the CDRRC catena consists of a mix of a short stoloniferous grass, *Scleropogon brevifolia*, with patches of tobosa grass, *Pleuraphis* (*Hilaria*) *mutica*. A comparison of the effects of *Aphaenogaster cockerelli* nests and *P. rugosus* nests on the soil protozoan community was made at the Nutt grassland site located at a mid-slope location on a gently sloping catena (<5%) approximately 8 km from the base of the mountains.

A soil extract was used in the most probable number (MPN) wells to approximate the soil solution properties of the study sites. The soil extract was prepared by mixing 200 g of soil from the sampling location in 1000 ml of distilled water. The mixture was heated at 60 °C for 2 h, filtered on Whatman #42, then autoclaved at 121 °C, 15 psi for 15 min. A dilution of 1:5 was used as the extract solution. Samples were homogenized by mixing 1 g of soil in 10 ml of soil extract in a Vortex mixer. Tubes were left for 30 min for sand sedimentation. After sedimentation, 1000 μl of the homogenate were transferred to the first row of a 24 cell culture plate previously filled with 900 μl of soil extract to make the first dilution of 1:10. The same procedure was used for the remaining dilutions with the final dilution of 1:1,000,000. Plates were incubated at 28 °C for 10–15 days. Protozoan counts were made by the most probable number method (Rodriguez-Zaragoza et al., 2005). Samples were examined for growth of amoebae and flagellates. We recorded the morphological forms of the amoebae as proposed by Anderson and Rogerson (1995) as follows: (type 1) flattened amoebae bearing sub-pseudopodia (like *Acanthamoeba*); (type 2) slender and cylindrical amoebae with a long non-eruptive pseudopodium (like *Hartmannella*); (type 3) eruptive triangular shape with a wide lobopodium (like Vahlkampfiidae); and (type 4) the fan-shaped amoebae (like Vannellidae and Platyamoebidae). Ciliates and testate amoebae were also recorded when observed in the wells. Total number of each morphological type of protozoa was obtained by the Thomas formula for MPN. Numbers were log-transformed and used for statistical analyses. Analysis of variance between soils from ant nests and reference soils was performed for protozoan types using SAS. Species–site relationships were evaluated by canonical correspondence analysis (Ludwig and Reynolds, 1988).

3. Results

Soil moisture was significantly higher in the *P. rugosus* nest disk soils and reference soils of the two lowest elevation sites than the soils from all other locations. Soils of the nest disks were significantly wetter than the reference soils ($p < 0.003$) (Fig. 1). Soil organic matter content was significantly higher on the two lowest elevation sites on the basin catena than the higher catena site soil and the piedmont soils (Fig. 1). Reference soils had significantly higher organic matter ($p < 0.002$) at the higher elevation site and nest soils had the highest organic matter content at the lowest elevation site ($p < 0.002$) (Fig. 1). Soil organic matter content was significantly higher in the low elevation, run-on sites on the Jornada than at the sites with sandy soils that are run-off areas ($p < 0.003$). There were no significant differences in soil organic matter between nest margin soils and reference soils in the remaining locations and no significance differences attributable to ant species at the remaining locations. There were no significant differences in soil moisture or soil organic matter with species or location at the Nutt grasslands sites (Fig. 2).

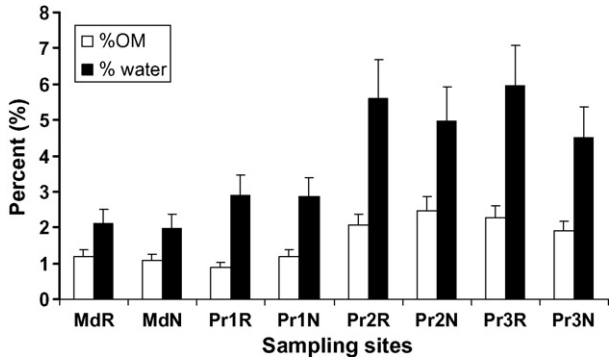

Fig. 1 – Percent soil moisture and percent soil organic matter in ant nest soils (N) and reference soils (R) on a creosotebush piedmont slope (MdR; MdN) and on a low sloping catena on a Chihuahuan Desert watershed (PrR; PrN). Pr1 is on sandy soil at the top of the catena; Pr2 is on loam soil at mid-slope of the catena; Pr3 is on clay-loam soil in the drainage basin of the catena. Md: *Myrmecocystus depilis* and Pr: *Pogonomyrmex rugosus*. The bars represent S.D. values.

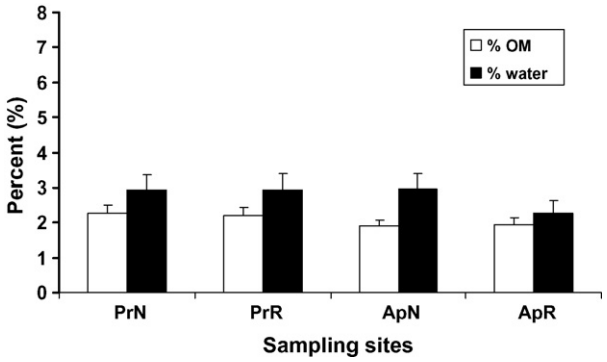

Fig. 2 – Percent soil moisture and percent soil organic matter in ant nest soils (N) and reference soils (R) associated with *Pogonomyrmex rugosus* (Pr) and *Aphaenogaster cockerelli* (Ac) at the Nutt grasslands. Means with S.D.

The least frequently encountered protozoans were the type 4 amoebae. Type 4 amoebae were only found in *A. cockerelli* nest soils at the Nutt grasslands and in the reference soils at the mid-slope location of the Jornada catena (Fig. 3). The most abundant protozoan, type 1 amoebae, were the only type extracted from the *P. rugosus* nest soils from the Jornada upper catena and from *M. depilis* nest soils on the piedmont (Fig. 3).

The reference soils at these locations supported both type 2 and type 3 amoebae (Fig. 3). There were no significant differences in type 2 amoebae among nest and reference soils and locations (Fig. 3). Type 3 amoebae made up a significantly higher proportion of the protozans counted at the Jornada *Erodium* site with significantly more type 3 amoebae in the reference soils ($p < 0.007$). Type 3 amoebae represented a higher proportion of the protozoans in the *A. cockerelli* nest soils in the Nutt grasslands ($p < 0.007$) than in the reference soils at this site.

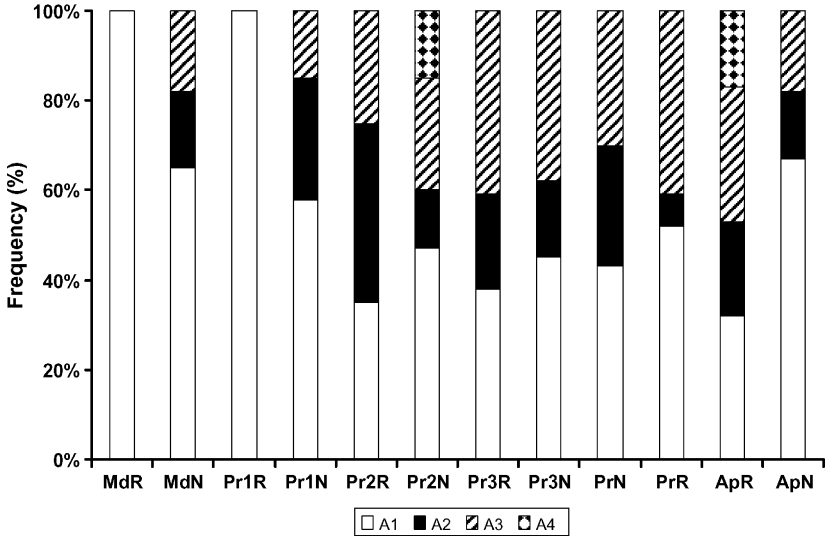

Fig. 3 – The frequency of amoeba types in soils associated with ant nests (N) and reference soils (R) on a shallow catena, on a low slope watershed in a grassland and on a piedmont slope of a watershed. Md: *Myrmecocystus depilis*—sandy soils, upper piedmont; Pr1: *Pogonomyrmex rugosus*—sandy-loam, top of catena; Pr2: *P. rugosus*—loam soils, mid-slope of catena; Pr3: *P. rugosus*—clay-loam soil, catena basin; PrN and PrR: *P. rugosus*—Nutt grassland; ApN and ApR: *Aphaenogaster cockerelli*—Nutt grassland. A1: flattened with sub-pseudopodia (like *Acanthamoeba*); A2: slender, cylindircal with long, non-eruptive pseudopodium (like *Hartmannella*); A3: eruptive triangular shape with wide lobopodium (like Vahlkampfiidae); A4: fan shape (like Vannellidae). Md: *Myrmecocystus depilis*—*Larrea tridentata* piedmont; Pr1, Pr2, Pr3: *Pogonomyrmex rugosus*—top, middle and basin of small catena, respectively; Pr and Ap: *Pogonomyrmex rugosus* and *Aphaneogaster cockerelli* in the Nutt grasslands.

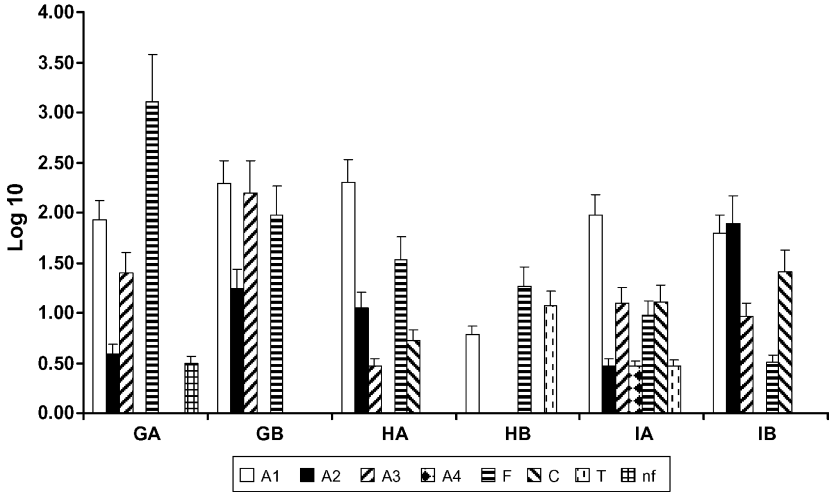

Fig. 4 – Comparison of the abundance (means with S.D.) of soil protozoans associated with nests (N) and reference soils (R) of two species of ants in different locations on a Chihuahuan Desert watershed. Md: *Myrmecocystus depilis*—sandy soils, upper piedmont; Pr1: *Pogonomyrmex rugosus*—sandy-loam, top of catena; Pr2: *P. rugosus*—loam soils, mid-slope of catena; Pr3: *P. rugosus*—clay-loam soil, catena basin. A: amoebae—A1: flattened with sub-pseudopodia (like *Acanthamoeba*); A2: slender, cylindircal with long, non-eruptive pseudopodium (like *Hartmannella*); A3: eruptive triangular shape with wide lobopodium (like Vahlkampfiidae); A4: fan shape (like Vannellidae). F: flagellates; C: ciliates; T: testate amoebae; nf: nanoflagellates.

Total protozoan numbers were significantly higher in *P. rugosus* nest soils at the top of the catena ($p < 0.001$) than in reference soils. There were no significant differences in total numbers of protozoans in the nest soils of *M. depilis* and *P. rugosus* at the two lower locations on the catena and the numbers of protozoans in the respective reference soils (Fig. 4). Protozoans were significantly more abundant in the *A. cockerelli* nest soils in the Nutt grasslands than in the reference soils ($p < 0.001$) but there was no significant difference in total numbers of protozoans in *P. rugosus* nest soils and reference soils in the Nutt grassland (Fig. 4). The *A. cockerelli* nest soils in the Nutt grasslands provided habitat for all of the types (8) of protozoans distinguished in this study. The soils associated with *M. depilis* nests and *P. rugosus* nests supported between 5 and 7 of the protozoan types (Fig. 4).

There were large qualitative and quantitative differences in protozoans associated with *M. depilis* nests on the Jornada piedmont slope (Fig. 4). Ciliates and nanoflagellates were isolated from *M. depilis* nest soils but not from *M. depilis* reference soils. There were significantly more flagellates in *M. depilis* nest soils than in reference soils ($p < 0.004$). There were similar differences in the protozoan assemblage between *P. rugosus* nest soils and reference soils at the upper catena (*Cryptantha*) site. Nest soil had no type 2 or 3 amoebae but did support testate amoebae which were absent from reference soils. Ciliates were isolated from reference soils but not from *P. rugosus* nest soils at this site. At the mid-slope site of the Jornada catena, the only notable difference in the protozoan assemblage in the *P. rugosus* nest soils and reference soils was the type 4 amoebae and testate amoebae isolated from the reference soils but absent in the nest soils (Fig. 4). At the lowest catenary position, the protozoan assemblage associated with *P. rugosus* reference soils included nanoflagellates which were absent from nest soils. There were significantly more flagellates isolated from reference soils than from nest soils at this site ($p < 0.004$) (Fig. 4).

There were both qualitative and quantitative differences in the protozoan assemblages associated with ant nest soils in the Nutt grassland (Fig. 5). *P. rugosus* nest soils had nanoflagellates which were absent from the reference soils. There were significantly more flagellates in *P. rugosus* nest soils than in reference soils ($p < 0.004$). Testate amoebae were found in

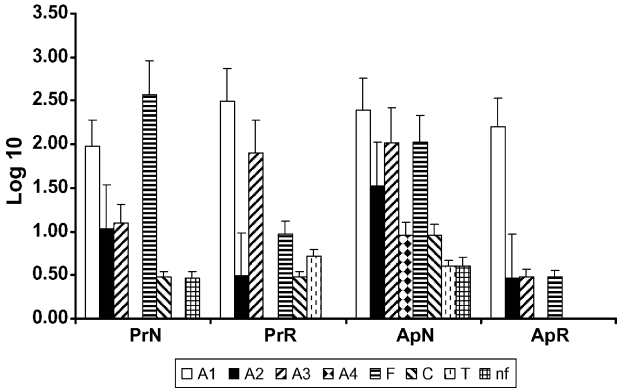

Fig. 5 – Comparison of the abundance (means with S.D.) of soil protozoans associated with nests (N) and reference soils (R) of two species of ants, *Pogonomyrmex rugosus* (Pr) and *Aphaenogaster cockerelli* in the Nutt grasslands. A: amoebae—A1: flattened with sub-pseudopodia (like *Acanthamoeba*); A2: slender, cylindircal with long, non-eruptive pseudopodium (like *Hartmannella*); A3: eruptive triangular shape with wide lobopodium (like Vahlkampfiidae); A4: fan shape (like Vannellidae). F: flagellates; C: ciliates; T: testate amoebae; nf: nanoflagellates.

the *P. rugosus* reference soils but not in the nest soils. The *A. cockerelli* nest soils in the Nutt grasslands provided habitat for all of the types (8) of protozoans distinguished in this study. No ciliates, testate amoebae or nanoflagellates were isolated from the *A. cockerelli* reference soils.

Canonical correspondence analysis ordinated the ant nest sites as a function of soil water content and organic matter. The organic matter and soil water content explained 21.4% of the variance in protozoan morphs in the ant nest soils. Ciliates and type 2 amoebae were correlated with water content and organic matter while type 3 amoebae were correlated with organic matter only. These patterns were applicable only to a subset of sites (Fig. 6). Type 1 amoebae and flagellates were weakly correlated with the soil water and organic matter. Testate amoebae, type 4 amoebae and nanoflagellates were not correlated with these soil factors (Fig. 6).

4. Discussion

The most probable number estimate described by Robinson et al. (2002) has been criticized because it fails to provide distinction between species that are encysted during sample preparation and species that were active at the time of sampling. Protozologists also suggest that the MPN procedure underestimates some groups of protozoans such as ciliates (Adl and Coleman, 2005). The question addressed by our study did not require distinction between active and encysted species of protozoans. We chose the MPN method because direct count methods are not useful in dry soils (Adl and Coleman, 2005) and we needed to compare the protozoan communities associated with desert ant nests with protozoan communities described by the MPN method that were reported for arid soils (Robinson et al., 2002; Rodriguez-Zaragoza et al., 2005). Despite the differences in methods, the protozoan assemblages described by Adl and Coleman (2005) for agricultural soils were similar to those found in desert soils in this study with very sparse ciliates and relatively abundant flagellates. Our study examined differences in the protozoan communities of soils modified by the nests of several species of ants compared with unmodified soils. For such a comparison, the MPN method, despite some technical problems, allows meaningful comparisons of ant nest soil protozoans and reference soil protozoans.

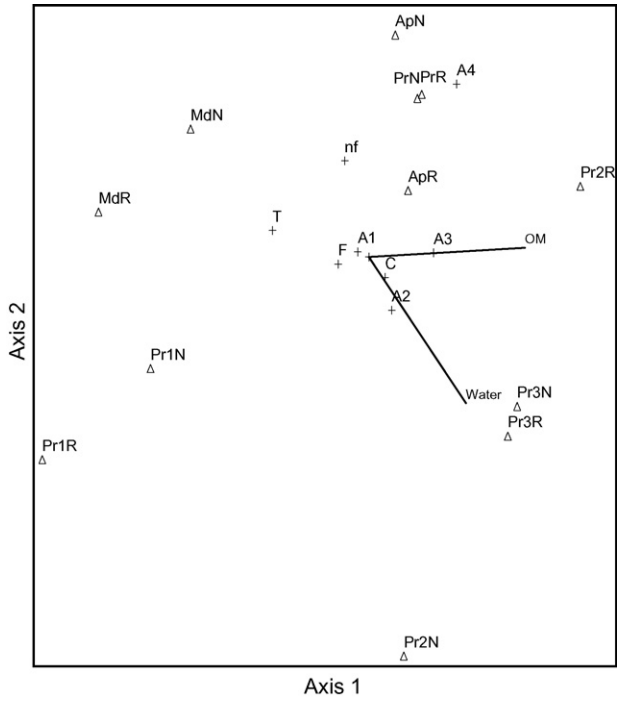

Fig. 6 – Canonical correlation ordination of protozoans in ant nest soils and reference soils from three locations in the Chihuahuan Desert. Md: *Myrmecocystus depilis*—sandy soils, upper piedmont; Pr1: *Pogonomyrmex rugosus*—sandy-loam, top of catena; Pr2: *P. rugosus*—loam soils, mid-slope of catena; Pr3: *P. rugosus*—clay-loam soil, catena basin. A: amoebae—A1: flattened with sub-pseudopodia (like *Acanthamoeba*); A2: slender, cylindircal with long, non-eruptive pseudopodium (like *Hartmannella*); A3: eruptive triangular shape with wide lobopodium (like Vahlkampfiidae); A4: fan shape (like Vannellidae). F: flagellates; C: ciliates; T: testate amoebae; nf: nanoflagellates.

The data from this study provided additional support for conclusions about the structure of protozoan communities in arid region soils (Varga, 1936; Parker et al., 1984; Rodriguez-Zaragosa and Garcia, 1997; Robinson et al., 2002). Soil protozoan assemblages in arid soils are dominated by naked amoebae with lower abundance of flagellates and nanoflagellates and sparse populations of testate (Testacea) amoebae and ciliates. With some notable exceptions, this was the structure of the protozoan communities in soils associated with the nests of three species of ants in a variety of locations on a Chihuahuan Desert watershed and Chihuahuan Desert grasslands. The restriction of flagellates to the sandy sites and to the nest soils on the mid-slope of the catena suggests that *P. rugosus* may have changed the relative proportion of sand in construction of the nests or may have selected sandier microsites for nest locations and that allowed for colonization by flagellates. There were no differences in the soil parameters that we measured in *P. rugosus* and *A. cockerelli* nest soils and reference soils at the Nutt grasslands that would explain the large differences in protozoan community structure in the *A. cockerelli* nest soils in comparison to the *P. rugosus* nest soils and/or the reference soils. Harvester ants (*P. rugosus*) have been reported to affect soil bulk density, soil temperature and percent pore space in addition to soil moisture and soil organic matter (Lei, 2000). The effects of ants on soil are dependent upon the nest building behavior of the ant species and on the characteristics of the soil (Whitford, 2002).

Since the nests of three species of ants included in this study (a seed harvester, a generalist, and a liquid feeder) had an effect on the soil protozoan community, we can conclude that the effects of ants on soil are not solely dependent on the food items carried to the nests by workers. The effects of ant nests on soils have been attributed to the chaff accumulations around the nest mounds of seed-harvesting ants (the shared characteristic of the ant species included in this study is nests that persist for a decade or longer; Chew, 1995; MacMahon et al., 2000). Continuous occupation of subterranean nests results in addition of fecal material to the soil, modification of

soil porosity by the construction of tunnels and chambers, and addition of uneaten items that are deposited around the nest mound. The protozoan communities associated with nest soils of the same ant species (*P. rugosus*) varied considerably in adjacent sites with different soils. The differences in protozoan community composition among sites were not directional or predictable based on soil texture or water run-off, run-on relationships. Based on these data, we can only conclude that the protozoan community associated with persistent ant nests varies both qualitatively and quantitatively among ant species, topographic positions and soil properties.

Soil modification by *M. depilis* and *P. rugosus* appeared to benefit the type 1 amoebae (*Acanthamoeba* type) which were the only amoebae in the soils associated with ant nests in the sandy-soil locations on the piedmont slope and highest elevation on the basin catena. Type 2 and type 3 amoebae in the reference soils at these two locations accounted for more than 30% of the total number of amoebae. *Acanthamoeba* type 1 amoebae were the most widely distributed and found in most of the samples from arid soils in Australia (Robinson et al., 2002). Our data show that the soils associated with these sites had the lower soil water content and organic matter than sites at lower elevations on the basin catena. The construction of tunnels and chambers by ants in the sandy soils of these sites may have affected soil microclimate and/or the patchiness of organic matter. A 10 cm diameter soil core cannot sample the fine scale heterogeneity that may result from the nest building and maintenance activities of ants.

The canonical correspondence analysis provided additional evidence that the primary factors affecting the protozoan communities in ant nest and reference soils were soil organic matter and soil water content. However, these soil parameters accounted for only 21.4% of the variance in types of protozoans from ant nest and reference soils. The canonical correspondence analysis suggests that all protozoan morphs will be present at sites with higher soil water content and higher organic matter. However, the quality of the organic matter may also be important for the establishment of several types of protozoans. The large variance in protozoan communities in ant nest and reference soils may be the result of differences in the composition of the microbial communities (species of bacteria and fungi) that differ among nests of different ant species and among nests of the same species at different topographic and/or geographic locations.

Soil modification by the activities of the three species of ants had important effects on the presence/absence and relative abundance of ciliates, flagellates, and nanoflagellates. The effects of soil modification by ants appear to be greater in the sandy soil environments than in the loam or clay-loam portions of the basin catena. This also suggests that ants may be modifying the soil microclimate or fine scale patchiness of soil water or organic matter. Studies that are confined to one area and topographic position may result in conclusions that are applicable only to that soil type and location. For example, Wagner et al. (1997) reported that amoebae and ciliates were more abundant in soils associated with the nests of the harvester ant, *Pogonomyrmex barbatus*, than in reference soils but flagellates were less abundant in nest soils than in reference soils. Topographic position and soil type affect those ecosystem processes that operate at the landscape scale such as run-off–run-on, sediment and organic debris transport and deposition. These processes interact with the soil properties that are modified by the activities of ants thereby modifying the soil environment as habitat for soil biota. Activities by ants that change the fine scale patchiness in the distribution and abundance of microbes may also have an effect on the suitability of that soil as habitat for protozoans.

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