available at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevier.com/locate/apsoil

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*

ARTICLE INFO

Article history:
Received 20 September 2006
Received in revised form
15 April 2007
Accepted 16 April 2007

Keywords:
Aphaenogaster cockerelli
Catena
Ciliates
Flagellates
Myrmecocystus depilis
Nanoflagellates

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).
0929-1393/$ – see front matter
doi:10.1016/j.apsoil.2007.04.002

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).