Biol Fertil Soils (2012) 48:227–233
DOI 10.1007/s00374-011-0619-4

ORIGINAL PAPER

Do long-lived ants affect soil microbial communities?

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Received: 15 November 2010 / Revised: 4 September 2011 / Accepted: 8 September 2011 / Published online: 27 September 2011

Abstract This study was designed to test the hypothesis that desert ant species that build nests that remain viable at a particular point in space for more than a decade produce soil conditions that enhance microbial biomass and functional diversity. We studied the effects of a seed-harvester ant, *Pogonomyrmex rugosus*, and two generalist ant species, *Aphaenogaster cockerelli* and *Myrmecocystus depilis*, on soil microbial communities. Microbial biomass was higher in *P. rugosus*-modified soils than in reference soils when soil water content was higher than 3%. Microbial biomass was either higher in reference soils or exhibited no difference in reference soils and nest-modified soils of *A. cockerelli* and *M. depilis*. There were differences in microbial functional diversity and microbial community level physiological profiles (MicroResp method) between ant-nest-modified and reference soils of the three ant species on some sampling dates. Temporal patterns of soil microbial communities associated with the ant species resulted from differences in soil moisture, density, and species composition of the annual plant communities associated with the ant nests and in reference areas. Differences in annual plant communities associated with ant nests and surrounding areas resulted in different chemical inputs into the soil organic-matter pools. This study shows that generalizations about the effects of long-lived ant nests on soil biota in arid regions must consider feeding behaviors of the ant species and temporal patterns of rainfall.

Keywords *Aphaenogaster cockerelli* · Climate · Community-level physiological profile (CLPP) · Functional diversity · Microbial biomass · MicroResp method · *Myrmecocystus depilis* · *Pogonomyrmex rugosus*

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Introduction

Animals are important in ecosystems primarily as agents that affect the structure and processes of ecosystems (Jones et al. 1994; Whitford 2002). In desert ecosystems, invertebrates affect ecosystem processes primarily by their effects on soil properties (Whitford 2000, 2001). Ants are abundant and conspicuous components of arid ecosystems, and some species are known to contribute to the heterogeneity or patchiness of landscapes (Whitford 2002). There are numerous reports that have documented the effects of seed-harvesting ants on the soils and on the vegetation around the margins of the nests (Boulton et al. 2003; Lei 2000; MacMahon et al. 2000; Wagner and Jones 2004; Whitford et al. 2008). Most published studies have focused on one species of seed-harvesting ant at a single location and in only one season. These studies have focused on large-body-size seed-harvesting ants because they produce large nests (>1 m in diameter), are central-place foragers, and accumulate organic matter and enrich soil nitrogen in the vicinity of the nests (Wagner and Jones 2004). The foraging behavior of seed-harvesting ants modifies soil in the vicinity of the nests, which results in increases in abundance and diversity of soil biota (Boulton et al. 2003; Wagner and Jones 2004).

In the northern Chihuahuan Desert, several species of ants construct nest mounds or circular clearings around the nest entrances. The surface structures of the nests are maintained by the ant colonies, some of which can survive for more than a decade (Chew 1995). Three species of Chihuahuan Desert ants with different foraging behaviors produce nests that persist for several years: *Pogonomyrmex rugosus*, a seed harvester, *Myrmecocystus depilis*, a liquid feeder–insect scavenger, and *Aphaenogaster* (*Novomessor*) *cockerelli*, a generalist that forages on seeds, plant parts, insects, and carrion (Chew 1995; Whitford et al. 1980). *A. cockerelli* ants deposit rejected forage materials at the edges of the nest mounds. We have recorded insect exoskeleton parts, dried flower petals, leaves and stems, and dung fragments that were deposited at the periphery of the nest mounds of *A. cockerelli* (Whitford et al. 1980). *M. depilis* nest mounds are characterized by a cemented surface. Cemented surfaces of ant nest mounds are produced by ants forming organo-mineral soil aggregates by combining organic fragments and mineral fragments with chemical secretions and excretory products (Cammeraat and Risch 2008). Based on published studies on the effects of seed-harvester ants on soil biota, we hypothesized that *P. rugosus*-nest-modified soils would support higher microbial biomass and higher diversity of microbial communities than nearby soils that were not affected by the ants. In addition, we hypothesized that these differences would be the same throughout the year.

Ant species that are honey-dew collectors and/or scavengers modify nest soils by the materials that are concentrated in the nest chambers or deposited as refuse at the periphery of the nest. The rates at which materials are concentrated and the quantity of material concentrated in and around nests depend upon several factors, including the longevity of nests at a particular point in space and the availability of carrion with respect to the daily and seasonal activity patterns of the ants (Bestelmeyer and Wiens 2003). Since the colonies of *A. cockerelli* and *M. depilis* remain active for a decade or longer, we hypothesized that the organic materials deposited as refuse or used in the construction of nest mounds produce soil conditions that result in increased biomass and diversity of the soil microbial communities.

Methods

We studied the soil microbial communities associated with the harvester ant *P. rugosus* at the base of a low-slope (<2%) catena at the Chihuahuan Desert Rangeland Research Center, New Mexico State University, located approximately 40 km NNE of Las Cruces, New Mexico, on the Jornada plain. The soil at the base of the catena is a clay-loam. The soil microbial communities associated with the nests of the liquid-feeding scavenger, honey-pot ant, *M. depilis*, were studied on a piedmont slope of a watershed at the Chihuahuan Desert Rangeland Research Center. Soil microbial communities associated with nests of the generalist–scavenger ant, *A. cockerelli*, were sampled on a low-slope catena (<2%) in the Nutt grasslands, approximately 50 km west of Hatch, New Mexico. The soil of the Nutt grassland catena is a silt–loam.

Soil samples were collected from five nests and five reference points of each ant species in December and January (cold winter season), May (hot-dry season), and August (hot-wet season). The ant nests to be sampled were the first five nests encountered walking from a random point in each habitat. Samples were collected with a 10-cm-diameter soil corer to a depth of 10 cm at the edge of the cleared nest disc and from a reference area 5 m from the nest margin. Soil samples were collected in plastic bags that were placed in an insulated container and taken to the laboratory. Soil samples were sieved through a 2-mm mesh, and 100 g of the sample was separated for microbial measurements.

Moisture was analyzed by drying samples at 105°C for 48 h and organic-matter content by burning samples at 490°C for 8 h (Steinberger et al. 1990). Microbial functional diversity and catabolic profiles were detected using the MicroResp™ plate (Chapman et al. 2007; Nannipieri et al. 2003). Fifteen different carbon sources of carbohydrates, carboxylic acids, amino acids, and aromatic carboxylic acids (25 µl each) were added to whole soil samples (0.32 g each) in deep well plates, yielding four replicates for each substrate in 96-well plates. Carbon dioxide evolution was measured by dye plates—a colorimetric reaction with absorbent alkali having the ability to measure carbon dioxide evolution. The plates were incubated at 27°C in the dark and read at 590 nm three times: immediately, after 6 h, and after 24 h. The results were calculated on the basis of the 16th well (water as substrate), which represented basal respiration. The Shannon–Weaver index (H') was used to determine microbial functional diversity:

$$H' = -\sum \mathrm{Pi}(\ln \mathrm{Pi})$$

where *Pi* is the ratio of the activity on a particular substrate and the sum of activities of all substrates (Zak et al. 1994).

Microbial biomass was also measured by dye plates. Water was added to whole soil samples in deep well plates covered by the dye plates in order to measure respiration. Glucose was added to determine microbial biomass according to the substrate-induced respiration method (Anderson and Domsch 1978). The metabolic index for CO_2 is a specific parameter for evaluating the effects of

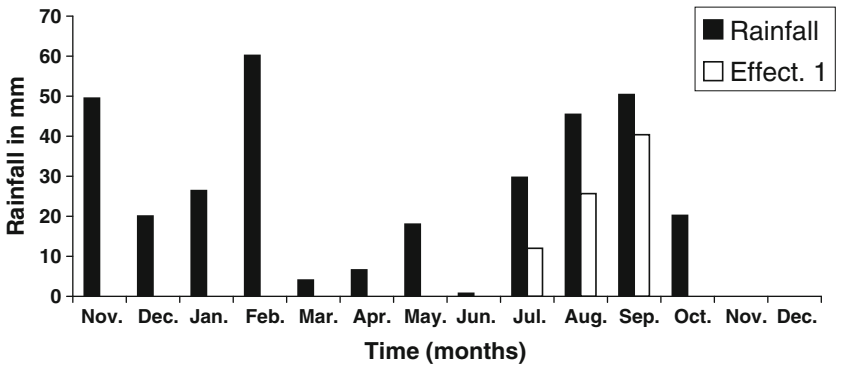

Fig. 1 Total monthly rainfall and effective rainfall (rain events>6 mm) during the hot summer months for the 2 months preceding the first sampling date through the duration of the study

environmental conditions on soil microflora (Anderson and Domsch 1990, 1993). All the data obtained in the study were subjected to statistical analysis of variance. Differences among means were examined by Duncan's test. Differences at the $p<0.05$ level were considered significant.

Results

Soil water content was significantly higher in January than on the other sampling dates because of the high antecedent rainfall—60.57 mm in November and December (Figs. 1 and 2). The low soil water content in May and August was the result of low antecedent rainfall in March and April and infrequent effective rainfall in July and August. Effective rainfall is defined as rain events of sufficient size to contribute to soil moisture. In the Chihuahuan Desert, where evaporation is extremely high in the hot summer months, effective rainfalls are rain events of greater than 6 mm. Soil water content was significantly higher in *P. rugosus*-nest-modified soils than in reference soils in the December samples. There were no differences in soil water contents between ant-nest-modified soils and reference soils for any of the other sampling dates. Soil organic-matter content was lower in the December samples than on the other sampling dates (Fig. 3). Organic-matter content was significantly higher in *M. depilis*-modified soils than in reference soils in the May samples but not on any of the other sampling dates.

There were significant differences in all of the soil microbial measures in ant-modified soils and in reference soils (Table 1).

Microbial biomass was higher in reference soils than in *M. depilis*-modified soils in the January, May, and December samples (Fig. 4). Microbial biomass was the same in *M. depilis*-nest and reference soils in August. Microbial biomass was not different in*A. cockerelli*-nest soils and reference soils on any of the sampling dates. Microbial biomass was higher in *P. rugosus* nest soils than in reference soils in the January and May samples and the same in nest-modified and reference soils in the August samples. Microbial biomass was significantly lower in *P. rugosus*-modified soils than in reference soils in the December samples. Microbial biomass at all locations was highest in the May samples and lowest in the August samples (Fig. 4).

Microbial respiration was higher in *P. rugosus*-nest soils than in reference soils in the January and May samples and lower than reference soils in the August samples (Fig. 5). There were no differences in respiration of *M. depilis*-nest and reference soils in the January and August samples, and it was higher in reference soils than in nest-modified soils in the May and December samples. Respiration of *A. cockerelli*-nest-modified soils was higher than of reference soils in May and August samples, with no differences between nest-modified and reference soils in December (Fig. 5).

The ratio of microbial biomass to total organic C is an indicator of microbial population growth on the organic matter at a location. The ratio of microbial biomass to organic matter was higher in *P. rugosus*-nest-modified soils than in reference soils only in January. The microbial biomass/organic-matter ratio was essentially the

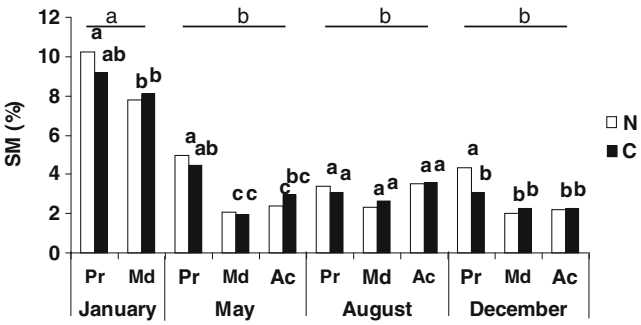

Fig. 2 Gravimetric soil water content (*SM*) associated with nest-modified soils (*N*) and surrounding reference soils (*C*) on the different sampling dates. *Pr P. rugosus*, *Md M. depilis*, *Ac A. cockerelli*. *Small letters* represent significant differences between the samples ($p<0.05$)

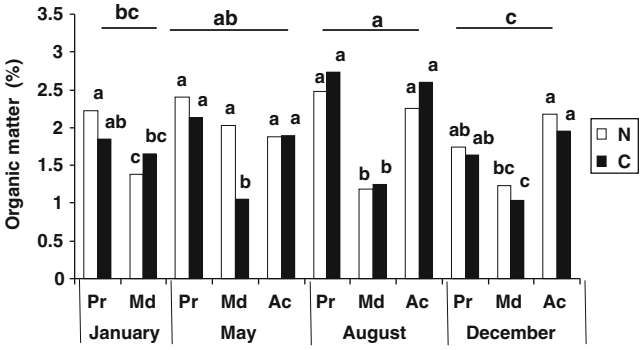

Fig. 3 Organic-matter content associated with nest-modified soils (*N*) and surrounding reference soils (*C*) on the different sampling dates. *Pr P. rugosus*, *Md M. depilis*, *Ac A. cockerelli*. *Small letters* represent significant differences between the samples ($p<0.05$)

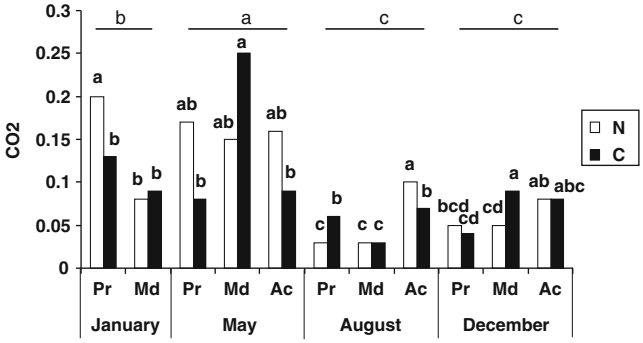

Fig. 4 Microbial biomass (*MB*) in nest-modified soils (*N*) and surrounding reference soils (*C*) on the different sampling dates. *Pr P. rugosus*, *Md M. depilis*, *Ac A. cockerelli*. *Small letters* represent significant differences between the samples ($p<0.05$)

same in nest-modified and reference soils at the other sampling times (Fig. 6). The microbial biomass/organic-matter ratio was much higher in reference soils than in *M. depilis* and *A. cockerelli*-nest-modified soils in May, but nearly equal in nest-modified and reference soils on the other sampling dates.

There was no consistent pattern of differences in functional diversity (*H*) for microbial communities in ant-nest-modified and reference soils (Fig. 7). Microbial functional diversity was higher in *P. rugosus*-modified soils than in reference soils in the January and May samples. Microbial functional diversity was higher in reference soils than in *P. rugosus*-modified soils in August but with no differences in nest soils and reference soils in the December samples. Microbial functional diversity was higher in reference soils than in *M. depilis*-modified soils in the January and December samples. Microbial functional diversity was higher in *M. depilis*-modified soils than in reference soils in the May samples. Microbial functional diversity was higher in *A. cockerelli*-nest soils than in reference soils in December but not different in nest-modified and reference soils on the other sample dates (Fig. 7).

There were seasonal patterns in differences in microbial community-level physiological profiles (CLPPs) between ant-nest-modified soils and reference soils (Fig. 8). The CLPP for all four groups of substrates was significantly higher in the *P. rugosus*-nest soils than in the reference soils in the January samples and equal in nest and reference soils in the May and August samples. In December, the microbial community in *P. rugosus*-nest soils had significantly higher metabolism of amino acid

Table 1 Univariate analysis of variance (ANOVA) for soil properties and microbial parameters as affected by month, treatment, and the interaction between them

	Month		Treatment		Month × treatment	
	F-test	*p* value	*F*-test	*p* value	*F*-test	*p* value
Soil properties						
Soil moisture (SM)	159.29	0.0001	11.21	0.0001	1.32	NS
Total organic carbon (TOC)	6.10	0.001	18.33	0.0001	3.14	0.0011
Microbial parameters						
CO_2	29.44	0.0001	3.57	0.0039	4.48	0.0001
Microbial biomass (MB)	35.97	0.0001	5.05	0.0002	5.85	0.0001
qCO_2	29.44	0.0001	3.57	0.0039	4.48	0.0001
MBOM	25.48	0.0001	13.33	0.0001	11.42	0.0001
Shannon–Weaver index (H')	16.53	0.0001	3.53	0.009	4.93	0.0001
Aromatic	13.62	0.0001	10.43	0.0001	5.77	0.0001
Carboxylic acids	13.82	0.0001	4.99	0.0002	7.09	0.0001
Carbohydrates	27.66	0.0001	1.54	NS	4.11	0.0001
Amino acids	1.63	NS	3.71	0.0069	0.94	NS
CLPP	7.60	0.0003	10.19	0.0001	4.12	0.0002

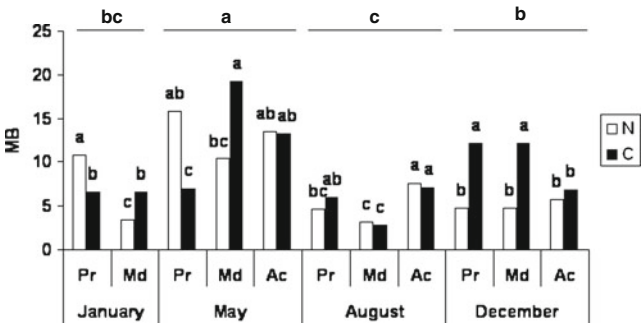

Fig. 5 Microbial respiration in nest-modified soils (*N*) and surrounding reference soils (*C*) on the different sampling dates. *Pr P. rugosus*, *Md M. depilis*, *Ac A. cockerelli*. *Small letters* represent significant differences between the samples ($p<0.05$)

substrates than the microbial community of the reference soils. The microbial community of the reference soils had higher metabolism of aromatic substrates than the microbial community in the *P. rugosus*-modified soils in the December samples. The CLPP was higher in *M. depilis*-nest-modified soils than in reference soils in the May and August samples, equal in the January samples, and higher in reference soils than in *M. depilis*-nest soils in December. There were no significant differences in CLPP between *A. cockerelli*-nest-modified and reference soils on any of the sampling dates (Fig. 8).

Discussion

The results of this study do not support the hypothesis that soils modified by long-lived ant colonies will support higher biomass and functional diversity of soil microbes than soils not affected by ant colonies. The absence of higher microbial biomass in the nest soils of the generalist–scavenger species, *A. cockerelli*, and the liquid-feeder–scavenger, *M. depilis*, suggests that the foraging and food handling behaviors of these species do not concentrate sufficient quantities of organic materials that are suitable substrates for an increase in microbial biomass or that the growth of the microbial populations was limited by other factors. Rainfall and soil moisture are frequently the most important limiting factors affecting microflora in arid and semi-arid systems (Chen et al. 2007; Jones and Wagner 2006; Wichern and Joergensen 2009). In a water-limited environment, differences in quality and quantity of substrates in ant-nest-modified and reference soils may not result in differences in microbial biomass.

Soil moisture was not the primary limiting factor for microbial biomass in *P. rugosus*-nest soils for the two sampling dates of the cool, low-evapotranspiration-rate season. The seed harvester-ant chaff accumulations at the margins of the nest discs provided the organic carbon source required for higher growth rates of the microbial populations. However, when effective rainfall was low or zero, the differences between microbial biomass in *P. rugosus*-nest soils and reference soils disappeared or were reversed. This pattern suggests that the positive effects of harvester ants on soil microbial biomass reported in previous studies (Boulton et al. 2003) may have been different or absent at other times of the year.

The higher microbial respiration in *A. cockerelli*-nest soils than in reference soils despite the lack of differences in microbial biomass, suggests differences in organic substrates in nest vs. reference soils. While microbial biomass and respiration of seed-harvester-ant, *P. rugosus*, nest soils were higher than of reference soils when gravimetric soil water content was higher than 4%, that difference disappeared when gravimetric soil water content dropped to less than 3%. Biomass and respiration were either equal or higher in reference soils than in ant-colony-

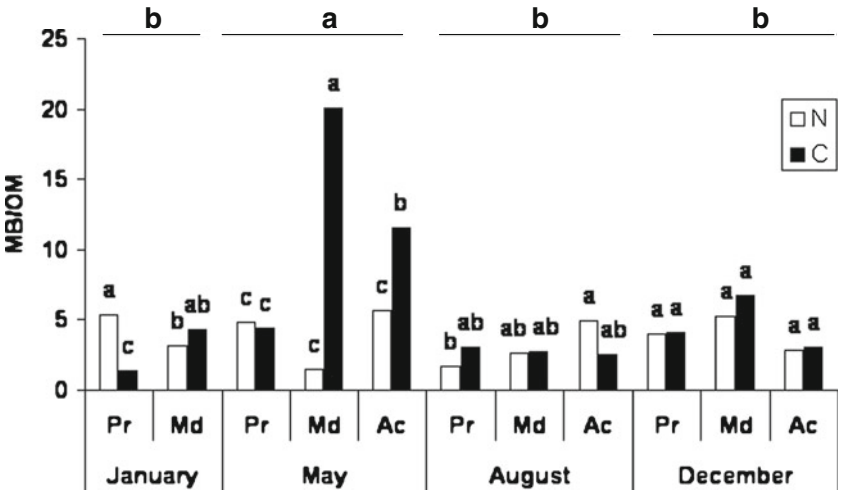

Fig. 6 The ratio of microbial biomass to soil organic matter in nest-modified soils (*N*) and surrounding reference soils (*C*) on the different sampling dates. *Pr P. rugosus*, *Md M. depilis*, *Ac A. cockerelli*. *Small letters* represent significant differences between the samples ($p<0.05$)

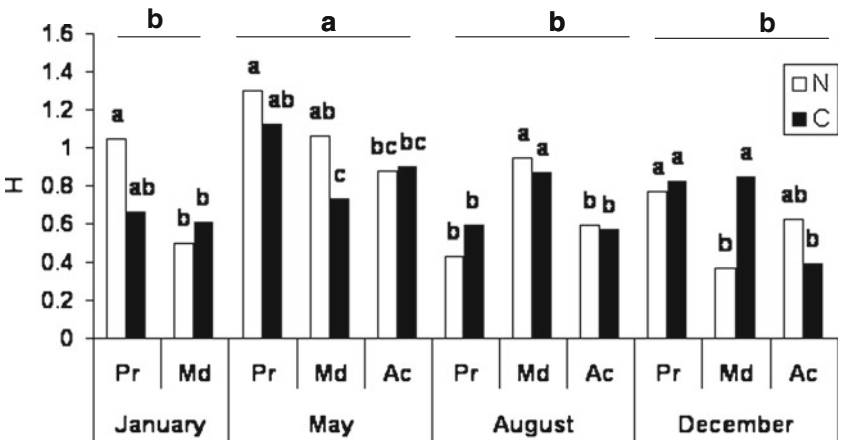

Fig. 7 Functional diversity index (*H'*) of the microbial communities in nest-modified soils (*N*) and surrounding reference soils (*C*) on the different sampling dates. *Pr P. rugosus*, *Md M. depilis*, *Ac A. cockerelli*. *Small letters* represent significant differences between the samples ($p<0.05$)

modified soils when gravimetric soil water content was 2% or less. Since the *P. rugosus* colonies were located in an area of high clay-content soils, microbial biomass and respiration were probably limited by low soil-water potential and not by substrate availability.

The differences in microbial functional diversity and microbial community-level physiological profiles between *A. cockerelli*- and *M. depilis*-nest-modified and reference soils suggest qualitative differences in the soil organic carbon between nest and reference soils despite a lack of quantitative differences in microbial biomass and microbial respiration. There were both qualitative (species composition) and quantitative (density and biomass) differences in spring and summer annual plants associated with the nests of *A. cockerelli* and *M. depilis* compared with reference areas (Whitford et al. 2008). Data from a greenhouse study demonstrated a relationship between microbial functional diversity and community-level physiological profiles with plant species and soil moisture (Chen et al. 2007). Since annual plant roots represent a major source of soil organic matter, the differences in microbial functional diversity and community-level physiological profiles are probably related to the chemical differences of the roots of the different species of annuals (Meier and Bowman 2008).

The results of this study clearly demonstrate that soil water availability is the most important factor affecting microbial biomass, microbial respiration, and functional diversity in arid ecosystems. The temporal patterns of differences in the microbial-community parameters in ant-nest-modified soils and in reference soils were largely attributable to soil moisture. However, the temporal patterns of microbial functional diversity and microbial community-level physiological profile differences between nest soils and reference soils resulted, in part, from the chemical nature of the organic matter inputs.

In conclusion, ant species that occupy nests at a single point in space for decadal time periods change the microbial communities in the nest-modified soils in qualitative and quantitative ways. While seed-harvester ants have the greatest effects on the soil microbial communities, other foraging types also produce soil modifications that affect microbial communities.

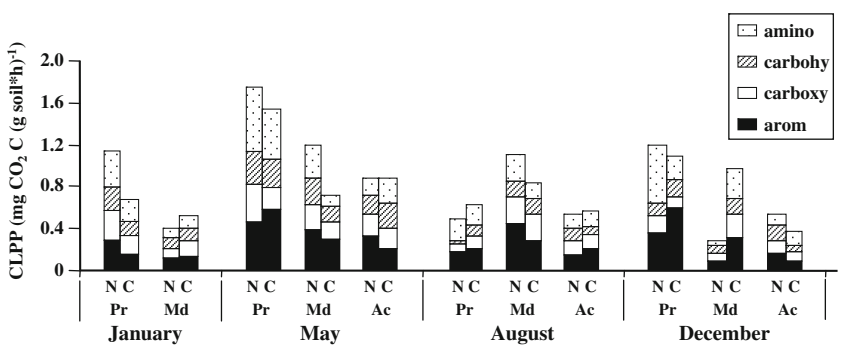

Fig. 8 Community-level physiological profiles (CLPPs) of the microbial communities in nest-modified soils (*N*) and surrounding reference soils (*C*) on the different sampling dates. *Pr P. rugosus*, *Md M. depilis*, *Ac A. cockerelli*

Acknowledgments Many thanks to Ms. S. Victor for her useful comments and help. This research was supported by Grant number Y410375-04R-22 from the International Arid Lands Consortium and is a contribution to the Jornada Long-Term Ecological Research Program.

We hereby declare that there is no conflict of interest related to this manuscript.

References

Anderson JE, Domsch KH (1978) Physiological method for quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221

Anderson TH, Domsch KH (1990) Application of eco-physiological quotients (qCO_2 and qD) on microbial biomass from soils of different cropping histories. Soil Biol Biochem 22:251–255

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem 25:393–395

Bestelmeyer BT, Wiens JA (2003) Scavenging ant foraging behavior and variation in the scale of nutrient redistribution among semi-arid grasslands. J Arid Environ 53:373–386

Boulton AM, Jaffee BA, Scow KM (2003) Effects of a common harvester ant (*Messor andrei*) on richness and abundance of soil biota. Appl Soil Ecol 23:257–265

Cammeraat ELH, Risch AC (2008) The impact of ants on mineral soil properties and processes at different spatial scales. J Appl Entomol 132:285–294

Chapman SJ, Campbell CD, Artz RRE (2007) Assessing CLPPs using MicroReSp (TM)—a comparison with biolog and multi-SIR. J Soils Sediments 7:406–410

Chen MM, Zhu YG, Su YH, Chen BD, Fu BJ, Marschner P (2007) Effects of soil moisture and plant interactions on the soil microbial community structure. Eur J Soil Biol 43:31–38

Chew RM (1995) Aspects of the ecology of 3 species of ants (*Myrmecocystus* spp., *Aphaenogaster* sp) in desertified grassland in Southeastern Arizona, 1958–1993. Am Midl Nat 134:75–83

Jones JB, Wagner D (2006) Microhabitat-specific controls on soil respiration and denitrification in the Mojave Desert: the role of harvester ant nests and vegetation. Western N Am Naturalist 66:426–433

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386

Lei SA (2000) Ecological impacts of seed harvester ants on soil attributes in a Larrea-dominated shrubland. Western N Am Naturalist 60:439–444

MacMahon JA, Mull JF, Crist TO (2000) Harvester ants (*Pogonomyrmex* spp.): their community and ecosystem influences. Annu Rev Ecol Syst 31:265–291

Meier CL, Bowman WD (2008) Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proc Natl Acad Sci U S A 105:19780–19785

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670

Steinberger Y, Shmida A, Whitford WG (1990) Decomposition along a rainfall gradient in the Judean Desert, Israel. Oecologia 82:322–324

Wagner D, Jones JB (2004) The contribution of harvester the ant nests, *Pogonomyrmex rugosus* (Hymenoptera, Formicidae), to soil nutrient stocks and microbial biomass in the Mojave Desert. Environ Entomol 33:599–607

Whitford WG (2000) Keystone arthropods as webmasters in desert ecosystems. In: Coleman DC, Hendrix PF (eds) Invertebrates as webmasters in ecosystems. CABI Publishing, New York, pp 25–41

Whitford WG (2001) Invertebrates: their effects on the properties and processes of desert ecosystems. In: Prakash I (ed) Ecology of desert environments. Scientific Publishers, Jodhpur, India, pp 333–356

Whitford WG (2002) Ecology of desert systems. Academic, London

Whitford WG, Depree E, Johnson P (1980) Foraging ecology of two Chihuahuan Desert ant species: *Novomessor cockerelli* and *Novomessor albisetosus*. Insect Soc 27:148–156

Whitford WG, Barness G, Steinberger Y (2008) Effects of three species of Chihuahuan Desert ants on annual plants and soil properties. J Arid Environ 72:392–400

Wichern F, Joergensen RG (2009) Soil microbial properties along a precipitation transect in Southern Africa. Arid Land Res Manage 23:115–126

Zak JC, Willig MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: a quantitative approach. Soil Biol Biochem 26:1101–1108