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Impacts of herbivorous insects on decomposer communities during the early stages of primary succession in a semi-arid woodland

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Abstract

Changes in nutrient inputs due to aboveground herbivory may influence the litter and soil microbial community responsible for processes such as decomposition. The mesophyll-feeding scale insect (*Matsucoccus acalyptus*) found near Sunset Crater National Monument in northern Arizona, USA significantly increases piñon (*Pinus edulis*) needle litter nitrogen (N) and phosphorus (P) concentrations by 50%, as well as litter inputs to soil by 21%. Because increases in needle litter quality and quantity of this magnitude should affect the microbial communities responsible for decomposition, we tested the hypothesis that insect herbivory causes a shift in soil microbial and litter microarthropod function. Four major findings result from this research: (1) Despite increases in needle inputs due to herbivory, soil carbon (C) was 56% lower beneath scale-susceptible trees than beneath resistant trees; however, soil moisture, N, and pH were similar among treatments. (2) Microbial biomass was 80% lower in soils beneath scale-susceptible trees when compared to resistant trees in the dry season, while microbial enzyme activities were lower beneath susceptible trees in the wet season. (3) Bacterial community-level physiological profiles differed significantly between susceptible and resistant trees during the dry season but not during the wet season. (4) There was a 40% increase in Oribatida and 23% increase in Prostigmata in susceptible needle litter relative to resistant litter. Despite these changes, the magnitude of microbial biomass, activity, and community structure response to herbivory was lower than expected and appears to take a long time to develop. These results suggest that herbivores impact soils in subtle, but important ways; we suggest that while litter chemistry may strongly mediate soil fertility and microbial communities in mesic ecosystems, the influence is lower than expected in this primary succession xeric ecosystem where season mediates differences in microbial populations. Understanding how insect herbivores alter the distribution of susceptible and resistant trees and their associated decomposer communities in arid environments may lead to better prediction of how these ecosystems respond to climatic change.

Keywords: Community-level physiological profiles; Enzyme activity; Insect herbivory; Insect-susceptible and resistant trees; Litter microarthropods; Microbial biomass; Piñon-juniper woodlands; Semi-arid

1. Introduction

Insect herbivores are common in terrestrial ecosystems (Schowalter, 2000), and can have a striking impact on the landscape by reducing plant biomass (Brown, 1994; Holland et al., 1996; Kosola et al., 2001), by altering the quality of litter inputs (Chapman et al., 2003), and by changing the microclimate beneath the area they infest (Classen et al., 2005). Altered microclimate and litter inputs should directly impact the processes that microbes mediate including decomposition (Chapman et al., 2003) and nitrogen (N) mineralization (Brown, 1994). Few studies, however, have investigated how these direct effects of herbivory may indirectly affect litter and soil microbial communities (Kuske et al., 2003). Investigating how insect herbivory modifies microbial populations and their functioning will lead to a better understanding of how species interactions may alter ecosystem processes.

Insect herbivores can indirectly change soil and litter microorganism populations in a variety of ways, but three

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mechanisms stand out. First, aboveground herbivory can increase the amount of herbivore frass and carcasses that enter an ecosystem (Hollinger, 1986; Risley, 1986; Lovett and Ruesink, 1995; Christenson et al., 2002). These products can be nutrient rich and may stimulate microbial populations. Second, aboveground herbivory can increase or decrease carbon (C) allocation between above- and below-ground biomass, thus altering the amount of substrate available for microbial growth (Dyer and Bokhari, 1976; Holland et al., 1996; Ritchie et al., 1998; Kosola et al., 2001). Third, herbivory can increase or decrease the quantity and quality of litter inputs by causing changes in the chemical properties of litter produced by plants (Risley and Crossley, 1993; Uriarte, 2000; Chapman et al., 2003), by changing the amount and timing of litterfall (Risley, 1986; Chapman et al., 2003) or, over the long-term, by causing a plant community shift from nutrient-rich to nutrient-poor plant species (Long et al., 2003).

Here, we examine how aboveground insect herbivory by the piñon needle scale (*Matsucoccus acalyptus*) seasonally alters soil and litter microbial communities beneath piñon pines (*Pinus edulis*) near Sunset Crater National Monument in northern Arizona, USA. This is an ideal system to examine this question for several reasons. First, Sunset Crater is dominated by piñon-juniper woodland, the third largest vegetation type in the US (West, 1984). The isolation of the dominant trees on this sparsely vegetated landscape provides an opportunity to examine the effects of herbivory with reduced interference from other plants and the effects of tree development on the soil environment. Second, there has been long-term monitoring and experimental removal for 16 years of the needle scale that chronically infest approximately 90% of the reproductively immature piñons (<60 years) at Sunset Crater (DelVecchio et al., 1993; Cobb and Whitham, 1998). Some trees at Sunset Crater are naturally resistant to the scale insect (hereafter 'resistant'), with scale populations never exceeding 0.1% of the populations found on susceptible trees. Other trees are naturally susceptible to the scale insect (hereafter 'susceptible'), and some trees have had scale insects manually removed for the past 16 years (hereafter 'removed'). Comparing scale-susceptible trees to scale-removed trees enables us to examine the short-term effects of herbivory (< 16 years), while comparing scale-susceptible trees to scale-resistant trees enables us to examine the long-term effects of herbivory (up to 60 years). Third, previous research at Sunset Crater has shown that scale herbivory reduces tree ring growth by 35% (Trotter et al., 2002) and increases needle litter N by 46%, litter phosphorus (P) concentrations by 55%, and needle litter inputs by 21%, while having no effect on needle litter lignin or condensed tannin concentrations relative to resistant trees (Table 1; Chapman et al., 2003). Increases in needle litter chemical quality of this magnitude should alter microbial populations and communities beneath susceptible trees. Fourth, DelVecchio et al. (1993); Gehring et al. (1997) have found that resistant trees have greater mycorrhizal colonization and increased root biomass compared to susceptible trees. An increase in mycorrhizae and root biomass beneath resistant trees would lead to a greater amount of soil C being shunted belowground, thus potentially increasing microbial populations by increasing C availability to decomposers (Wardle et al., 2002). Finally, insect populations are expected to expand their range into previously uninhabited forests with global climatic change (Dale et al., 2001). Range expansions should be expected in arid climates first because plant populations in these ecosystems are often functioning at their physiological extremes and these ecosystems are, therefore, less buffered to insect expansions. Understanding how insect herbivores alter seasonal decomposer communities in arid environments may lead to models that better predict how ecosystems will respond to future climatic change.

Table 1
Needle litter chemistry needle litter inputs and fine root production

	Susceptible	Resistant	Removed
C:N[a]	64.2a	99.9b	106.2b
Phosphorus (g kg^{-1})[a]	1.7a	1.1b	1.1b
Nitrogen (g kg^{-1})[a]	7.9a	5.4b	5.0b
Lignin (%)[a]	18.2a	19.1a	17.2a
Lignin:Nitrogen[a]	23.9a	35.5b	36.0b
Tannin (g kg^{-1})[a]	51.3a	49.8a	50.3a
Needle inputs (g m^{-2} yr^{-1})[a]	90.6a	75.1b	76.0b

Within rows, contrasting letters denote significant differences among treatments (scale-susceptible, resistant, and scale-removed trees).
[a] Data from Chapman et al. (2003).

In this paper, we ask three explicit questions about the impact of insect herbivory on decomposer communities: (1) Does aboveground insect herbivory affect soil and litter microorganisms? (2) How does season mediate the effects of herbivores on soil microorganisms? (3) Do herbivore effects on microorganisms take a long time to develop?

2. Materials and methods

2.1. Site description

This project was conducted near Sunset Crater National Monument on the Colorado Plateau in northern Arizona (35°22′N, 111°33′W) during the summer of 2002. Sunset Crater erupted in 1064 AD, covering 800 km^2 of the landscape with a thick layer of ash and cinders (Hooten et al., 2001). Soils at the site are classified in the Soil Taxonomic family Typic Ustorthents. Sunset Crater is a nutrient-limited and dry piñon-juniper woodland (Cobb et al., 1997; Swaty et al., 1998). Piñons are the dominant woody plant at our study site followed by one-seed juniper (*Juniperus monosperma*) and apache plume (*Fallugia paradoxa*). The vegetation at this study site is widely spaced with inter-crown areas that are mostly vegetation free.

2.2. Experimental design

For the past 16 years, scale infestation has been prevented on a group of trees by annually removing egg masses found at the base of susceptible trees (Trotter et al., 2002). In total, we had four treatment categories: scale-susceptible, scale-resistant,

scale removed, and inter-crown areas. Eight inter-crown areas devoid of vegetation were sampled to assess the influence of tree crowns on soil microbial communities. We randomly selected 12 trees that were susceptible to scale infestation, 12 trees that were naturally resistant to infestation, and 12 trees that were susceptible to infestation but from which the insects had been experimentally removed. The designation of trees as being either resistant or susceptible to scale attack is based upon long-term studies showing that adjacent trees differ dramatically in their scale populations and experiments in which scales were transferred to both tree groups and their survival recorded (Cobb and Whitham, 1993). These studies found that scale mortality was 3.4 times greater on trees that supported low scale loads than on trees with high scale loads, which argues that trees differ significantly in the resistance to scale attack (Gehring et al., 1997). Comparisons between scale-susceptible and removed trees enable us to examine short-term (<16 years) responses of soil properties to herbivory. Comparisons between scale-susceptible and resistant trees allow us to examine long-term (>60 years) responses of soil properties to herbivory. Both of these comparisons are important for understanding the response of soil communities to herbivory, as soil properties are likely to vary in their rate of response to herbivore treatments.

2.3. Soil Sampling

Beneath each tree, four mineral soil samples (0–10 cm, approximately 600 g) were taken midway between the trunk and the crown dripline in each of the four cardinal directions using a hand trowel. Four soil samples were also randomly collected in each inter-crown area at least one crown length away from the dripline. Soil samples were composited and kept cool (4 °C) until they were processed (within 48 h). Samples were collected in hot and dry June prior to the monsoon rains (hereafter 'pre-monsoon'), in warm and moist August after the monsoon rains (hereafter 'post-monsoon'), and in cool and moist November (hereafter 'November') to assess how seasonal changes in soil moisture and temperature alter microbial populations in the various treatments.

2.4. Soil characteristics

Total soil C and N were determined on air-dried soils that had been ground to a fine powder by hand with a mortar and pestle and run on a Carlo-Erba Model 2500 CN elemental analyzer (Milan, Italy). Soils were sieved (2 mm) prior to soil analyses. Soil pH was determined using a 1:2 suspension of air-dry soil to 0.01 M $CaCl_2$ solution (Orion 720A pH Meter, Allometrics, Inc., LA, USA). Soil C, N, and pH were measured for the pre-monsoon soil collection only as we did not expect these parameters to change during the short duration of the study. Soil gravimetric water content (GWC) was determined for each sample (105 °C) and all data are expressed on an oven-dry mass basis.

2.5. Microbial biomass-N

We measured microbial biomass-N using a modification of the fumigation–extraction method described by Haubensak et al. (2002). One 30 mL ($\sim$17–26 g), sieved, field-moist subsample was immediately extracted with 50 mL of 0.5 M K_2SO_4 by shaking for 1 h, then filtered through a Whatman No. 1 filter paper previously leached with (100 mL) deionized water. A separate subsample was extracted and filtered after it had been fumigated in a vacuum desiccator with hydrocarbon-stabilized $CHCl_3$ for 5 days. Twenty milliliter aliquots of extracts were digested using a modified micro-Kjeldahal digestion and analyzed for total N on a Lachat AE Flow Injection Auto-analyzer (Lachat Instruments, Milwaukee, WI, USA). The total N in the unfumigated samples was subtracted from the total N in the fumigated samples to calculate chloroform labile-N. A K_{EN} correction factor of 0.2 was used to estimate biomass-N from chloroform labile-N (Davidson et al., 1989). Microbial biomass-N was measured pre-monsoon rains and again post-monsoon rains to account for seasonal changes in microbial populations.

2.6. Enzyme assays

To estimate if microbial activity and function was affected by increased nutrient availability beneath susceptible trees, eight ecologically relevant enzymes were assayed: β-1,4-glucosidase, α-1,4-glucosidase, β-galactosidase, β-xylosidase, cellobiohydrolase, *N*-acetyl-glucosaminidase (NAGase), alkaline phosphatase, and sulfatase. Enzymes were measured using the MUB-linked substrates 4-methylumbelliferyl β-D-glucosidase, 4-methylumbelliferyl-α-D-glucoside, 4-methylumbelliferyl β-D-galatoside, 4-methylumbelliferyl 7-β-D-xyloside, 4-methylumbelliferyl β-D-cellobioside, 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide, 4-methylumbelliferyl phosphate disodium salt, and 4-methylumbelliferyl sulfate potassium salt, respectively. The first five enzymes assist with the breakdown of energy sources such as carbohydrates and polysaccharides into smaller components that are more readily available for uptake by soil organisms (O'Connell, 1987; Eivazi and Tabatabai, 1988; Sinsabaugh, 1994; Eivazi and Bayan, 1996; Boerner et al., 2000). *N*-acetyl-glucosamindase is a chitinolytic enzyme that is involved in the mineralization of N from chitin (Olander and Vitousek, 2000). Phosphatase breaks ester linkages and is involved in the release of inorganic P (Eivazi and Tabatabai, 1977; Tarafdar et al., 1989). Sulfatase breaks ester linkages and is involved in the release inorganic forms of sulfur (Ganeshamurthy and Nielsen, 1990; Eivazi and Bayan, 1996). Enzymes were measured in the pre-monsoon, the post-monsoon, and November season to assess how microbial activity may respond to seasonal changes in soil moisture and temperature.

Enzymes were assayed following the methods outlined in Boyle et al. (2005). Briefly, field-moist soil (1 g) was suspended in 100 mL of 5 mM bicarbonate buffer solution (pH 8.2). Microtiter plate test wells were inoculated with 100 μL of the suspended soil solution and 100 μL of an enzyme

substrate solution; this was repeated until all enzyme substrates were represented six times on a single plate (Sinsabaugh et al., 1991). Quenching standards were also included on each plate. Following inoculation, plates were read using a Fluoromax fluorometer (Jobin Yvon-Spex, Edison, NJ, USA) with an attached MicroMax Microwell plate reader (excitation of 360 nm, emission 450 nm). Plates were incubated for 1 h at 27 °C and read again on the fluorometer. Enzyme activities were calculated as μm product kg soil^{-1} h^{-1} and then were divided by calculated soil C in order to express the values per unit of soil C (μm product kg soil C^{-1} h^{-1}).

2.7. Community-level physiological profiles

In order to assess how herbivory alters bacterial and fungal communities, we determined community-level physiological profiles (CLPP) using microtiter plates prepared by Biolog, Inc. (Hayward, CA, USA). These plates contain C sources that are commonly used to metabolically 'fingerprint' bacterial and fungal communities (Garland and Mills, 1991). Bacterial CLPPs were assessed using ECO plates that contain 31 environmentally relevant C substrates replicated three times per plate; fungal CLPPs were assessed using SFN-2 plates that consist of 95 unique C substrates. Plates were prepared following methods outlined in Classen et al. (2003).

Well-absorbance values less than or equal to 0.06, the detection limit of the spectrophotometer, were set to zero. Bacterial plate replicates were averaged and the 750 nm values (turbidity only) were subtracted from the 590 nm values (color development plus turbidity) to denote activity in each well (Garland and Mills, 1991). Fungal values were calculated using the 750 nm values (turbidity; Buyer et al., 2001). Data were normalized to reduce the influence of differences in inoculation densities for bacterial and fungal plates by dividing the color or turbidity development of each well by the total color or turbidity development of the entire plate (Garland, 1996; Classen et al., 2003). CLPPs were measured pre-monsoon and post-monsoon rains in order to assess seasonal driven changes in microbial communities.

2.8. Litter microarthropods

To test how litter microarthropod communities differed among treatments, litter samples ($n=12$, total 36 samples) were collected from scale-susceptible, scale-resistant, and scale-removed trees in August of 2002. Four litter samples (O horizon) were taken from beneath each tree in each of the cardinal directions using a PVC tube (8.89 cm diameter) and hand trowel. Samples were collected midway between the trunk and the crown dripline and homogenized within each tree. Immediately after returning from the field, samples were transferred to Tullgren funnels modified to extract microarthropods (Santos et al., 1978). Samples were extracted for 48 h into water filled containers. Collected samples were preserved in a solution of 95% ethanol and 5% DI water. We limited our microarthropod sorting to 10 scale-susceptible and 10 scale-removed trees. All microarthropods were counted and identified to morphospecies by an expert in oribatid mites (Karen LaMoncha at Humboldt State University, CA, USA). Oribatid mites are important components of the litter and soil food web in arid environments and were therefore sorted to the species level. The reference collection for these organisms will be kept at NAU within the Department of Biological Sciences.

2.9. Statistical analysis

One-way analyses of variance (ANOVAs) were used to test for the effects of the four treatments on soil characteristic (%C, %N, C:N, pH), pre- and post-monsoon GWC, pre- and post-monsoon microbial biomass, and individual enzyme differences among treatments. When the ANOVA models indicated a significant treatment effect, a post hoc Tukey's honestly significant difference (HSD) test was conducted to determine differences among treatment means. Soil %C and %N data were arcsine-transformed and microbial biomass and all the enzyme data were log-transformed to improve normality. Non-transformed data are shown in all tables and figures. Statistics were conducted with JMP 5 statistical software with significance defined as $p \leq 0.05$ (SAS Institute, 2001, Pacific Grove, CA).

Soil bacterial CLPPs, soil fungal CLPPs, and litter microarthropod communities were analyzed with PC-ORD 4 (Mjm Software, Glendale, OR). Multi-response permutation procedures (MRPP) were used to test for differences in bacterial, fungal, and microarthropod responses among treatments (McCune and Grace, 2002). Between-group homogeneity is described by the 'A' statistic. When groups are not different from each other, the 'A' statistic is zero; if they are completely distinct from each other the 'A' statistic is equal to one (McCune and Grace, 2002). We accepted 'A' values of 0.03 with a $p < 0.01$ as being the lowest acceptably significant value. Graphs were generated using non-metric multi-dimensional scaling (NMDS) (McCune and Grace, 2002). The pre-monsoon and post-monsoon seasons were tested separately.

3. Results

3.1. Effects of herbivory on soil chemistry

Scale-susceptible trees had 56% less soil C than resistant trees (Table 2, $p < 0.0001$), however, there were no differences in soil C between scale-removed and susceptible trees. Inter-crown areas had also 87% less soil C than soil beneath tree crowns (Table 2, $p < 0.0001$). There were no differences in soil N among scale-susceptible, resistant, and scale-removed trees (Table 2). Inter-crown soils had 84% less N than soil beneath tree crowns ($p < 0.0001$).

Soil pH, C:N, and GWC were similar among scale-susceptible, resistant, and scale-removed trees (Table 2). GWC increased beneath tree crowns by 192% between the pre-monsoon and the post-monsoon season, and by 9% between the post-monsoon season and November (data not shown, $p < 0.001$).

Table 2
Effects of herbivory on soil chemical characteristics and gravimetric water content (GWC) (0–10 cm)

Soil characteristics	Susceptible	Resistant	Removed	Inter-crown
PH	6.70a (0.10)	6.63a (0.11)	6.90a (0.41)	6.65a (0.03)
Soil nitrogen (%)	0.09a (0.01)	0.20a (0.04)	0.13a (0.03)	0.02b (>0.01)
Soil carbon (%)	1.43a (0.22)	3.26b (0.77)	1.92ab (0.40)	0.28c (0.05)
Soil C:N	15.38a (0.48)	16.15a (0.48)	14.65ab (0.41)	12.74b (0.86)
% GWC June	0.7a (0.1)	4.4b (2.9)	0.9a (0.2)	0.3a (0.1)
% GWC August	3.4a (0.4)	6.6a (1.3)	5.0a (0.7)	10.4a (5.2)
% GWC November	6.0a (0.6)	8.9ab (0.9)	6.9ab (0.7)	10.0b (1.2)

Soil pH, %N, %C and C:N were determined in June. Standard errors are shown below the means in parentheses ($n=12$). Within rows, contrasting letters denote significant differences among treatments (scale-susceptible, resistant, and scale-removed trees and inter-crown areas) using a Tukey's HSD test ($p<0.05$).

3.2. Effects of herbivory on microbial biomass-N

Scale-susceptible trees had 80% lower soil microbial biomass than resistant trees in the pre-monsoon season (Fig. 1, $p=0.0018$). Pre-monsoon microbial biomass beneath scale-removed trees was intermediate between susceptible and resistant trees. Differences in the pre-monsoon season were erased in the post-monsoon season where microbial biomass in soils beneath scale-susceptible, resistant, and removed trees was similar (Fig. 1). Microbial biomass was significantly lower in inter-crown areas when compared to soil beneath tree crowns in both pre- and post-monsoon seasons ($p<0.0001$).

3.3. Effect of herbivory on soil enzymes

There was large variation in enzyme rates within and among treatments. Scale-susceptible trees had lower β-galactosidase and *N*-acetyl-glucosaminidase activity in soil than resistant trees during the post-monsoon season (Table 3, $p=0.007$ and 0.015, respectively). There were no differences among scale-susceptible, resistant, and scale-removed microbial enzyme activity in the pre-monsoon or November seasons (Table 3).

Inter-crown areas had significantly higher β-1,4-glucosidase, α-1,4-glucosidase, β-galactosidase, β-xylosidase,

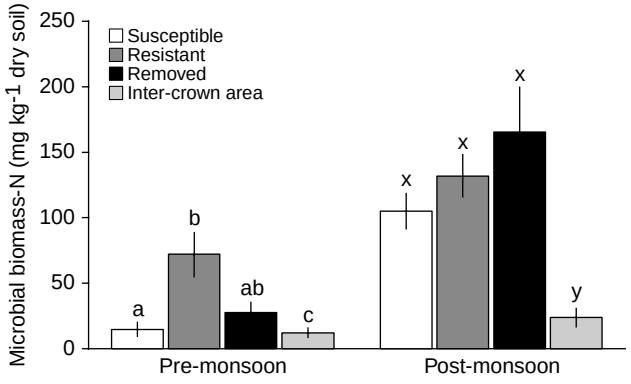

Fig. 1. Mean microbial biomass beneath scale-susceptible, resistant, and scale-removed trees and adjacent inter-crown areas during pre- and post-monsoon seasons ($n=12$). Letters denote significant differences using a Tukey's HSD test ($p\leq0.05$); error bars are ±1 SE.

Table 3
Effect of herbivory on enzyme activities in soil during three seasons (pre-monsoon, post-monsoon, and November)

Enzyme	Susceptible	Resistant	Removed	Inter-crown
Pre-monsoon				
Ligno-cellulolytic enzyme activities				
α-1,4-glucosidase	326a (597)	19a (19)	346a (287)	4140a (2315)
β-1,4-glucosidase	579a (246)	146a (73)	779a (417)	2315a (1431)
Cellobiohydrolase	192a (108)	9a (9)	197a (186)	1411a (1130)
β-galactosidase	213a (122)	7a (6)	229a (209)	1777a (1213)
β-xylosidase	171a (104)	11a (10)	218a (201)	1543a (1172)
Nutrient mineralizing enzyme activities				
N-acetyl-glucosaminidase	237a (142)	79a (66)	228a (208)	1705a (1287)
Alkaline phosphatase	479a (211)	66a (35)	236a (187)	2780a (1955)
Sulfatase	84a (83)	0a (0)	90a (90)	782a (493)
Post-monsoon				
Ligno-cellulolytic enzyme 0activities				
α-1,4-glucosidase	1b (1)	303ab (123)	781ab (544)	12,536a (5675)
β-1,4-glucosidase	241b (166)	2904ab (1419)	3335ab (1998)	2991a (1428)
Cellobiohydrolase	0b (0)	331ab (129)	541ab (370)	2448a (1217)
β-galactosidase	0b (0)	401a (164)	783ab (501)	2955a (1605)
β-xylosidase	0b (0)	369ab (133)	898ab (574)	2849a (1206)
Nutrient mineralizing enzyme activities				
N-acetyl-glucosaminidase	35b (35)	1296a (751)	997ab (546)	3941a (1488)
Alkaline phosphatase	455a (399)	10,805a (6842)	5106a (2727)	24,501a (8955)
Sulfatase	19a (13)	17a (14)	121a (121)	702a (400)
November				
Ligno-cellulolytic enzyme activities				
α-1,4-glucosidase	306a (154)	171a (87)	576a (279)	6235a (3497)
β-1,4-glucosidase	1451a (365)	1004a (333)	1201a (286)	4753a (2838)
Cellobiohydrolase	238a (125)	153a (73)	408a (238)	3358a (1856)
β-galactosidase	216a (122)	104a (51)	520a (292)	4324a (2493)
β-xylosidase	265a (139)	199a (85)	511a (244)	3862a (2212)
Nutrient mineralizing enzyme activities				
N-acetyl-glucosaminidase	464a (155)	264a (83)	265a (97)	3093a (1758)
Alkaline phosphatase	400a (142)	320b (110)	544ab (197)	3094a (1920)
Sulfatase	60ab (42)	1.0a (0.7)	190a (119)	2112b (1328)

Standard errors are shown below the means in parentheses ($n=12$). Activity units for enzymes are μmol kg^{-1} soil C h^{-1}. Within rows, contrasting letters denote significance among treatments (scale-susceptible, resistant, scale-removed, and inter-crown) using a Tukey's HSD test ($p<0.05$).

cellobiohydrolase, and *N*-acetyl-glucosaminidase activity when compared to scale-susceptible trees in the post-monsoon season (Table 3, $p=0.023$, 0.016, 0.009, 0.005, 0.018, 0.015, respectively). Inter-crown areas also had greater sulfatase activity than soils beneath scale-resistant and scale-removed trees (Table 3, $p=0.039$) in the November season.

3.4. Effects of herbivory on soil bacterial and fungal CLPPs

Scale-susceptible trees had different soil bacterial CLPPs when compared with resistant trees in the pre-monsoon season (Fig. 2A, Table 4). Scale-removed trees had similar bacterial CLPPs when compared with susceptible trees and when compared with resistant trees. Significant effects among treatments were absent in the post-monsoon season, where soil bacterial CLPPs were similar beneath susceptible, resistant, and scale-removed trees (Fig. 2B, Table 4).

Scale-susceptible trees had distinct fungal CLPPs when compared to resistant trees during the pre-monsoon season (Table 4). Scale-removed trees were similar to both susceptible and resistant trees during the pre-monsoon season; and differences among treatments were absent in the post-monsoon season. Fungal CLPPs were significantly different between soils located in inter-crown areas and those located beneath tree crowns (Table 4) in the post-monsoon season.

3.5. Effect of herbivory on litter microarthropods

We found significant differences in the microarthropod community beneath scale-resistant and susceptible trees. Eighteen morphospecies of microarthropods and 20 species of Oribatid mites were identified across all treatments. Litter microarthropod and oribatid mite community composition differed between scale-susceptible and resistant trees (Fig. 3A and B, $p=0.018$, 0.015, respectively). Twelve taxa of litter microarthropods and 10 species of oribatid mites were found from all sorted samples (Table 5). Fungivores (Collembola and Oribatida) and microphytophadus (Prostigmata) were the dominant organisms found in needle litter at Sunset Crater (Table 5). While there were no differences in litter microarthropod or oribatid mite abundance between scale-resistant and susceptible trees (data not shown), there was a 40% increase in Oribatida and a 23% increase in Prostigmata in needle litter beneath scale-susceptible crowns. Additionally, the total number of microarthropods found beneath scale-susceptible crowns (687 microarthropods) was greater than resistant crowns (487 microarthropods).

4. Discussion

Our study adds to the scant literature on the influence of insect herbivory on microbial populations (Bardgett et al., 1998). Previous work at our study site has shown that insect herbivory increases litter inputs and litter quality (Chapman et al., 2003), soil temperature and soil moisture (Classen et al., 2005), and decomposition rates (Chapman et al., 2003), suggesting a strong influence on soil and litter microorganism communities. The present study demonstrates that above-ground insect herbivory can also significantly alter microbial populations, but that this effect is mediated by season and may require a long time to develop in this semi-arid environment.

Herbivory decreased soil C (Table 2), despite significant increases in needle litter inputs (Table 1), and it did not affect total soil N. In addition, herbivory by the scale insect decreased microbial biomass-N (Fig. 1) and altered bacterial CLPPs (Fig. 2) during the dry, pre-monsoon season, while decreasing soil enzyme activities (Table 3) during the relatively moist, post-monsoon season. Herbivory also significantly altered litter microarthropod community composition (Fig. 3), and this influence appeared to be mediated by changes in soil temperature and moisture. Taken together, these results suggest that soil abiotic conditions may be more important drivers of

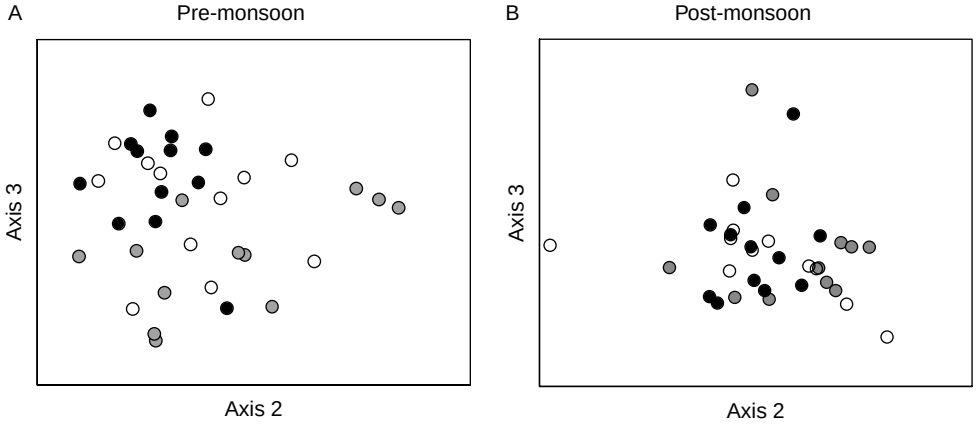

Fig. 2. Bacterial community-level physiological profiles (CLPPs) among scale-susceptible (gray circles), resistant (black circles), and scale-removed (open circles) trees during pre-monsoon (A) and post-monsoon (B) seasons ($n=12$). Scale-susceptible and resistant trees differed significantly in the pre-monsoon, but not post-monsoon season (see Table 4). Each point represents the CLPP of soil beneath an individual tree. Analyses were conducted using multi-response permutation procedures (MRPP).

Table 4
Herbivore effects on bacterial and fungal community level physiological profiles (CLPP) during the pre-monsoon (A, C) and post monsoon (B, D) seasons

		A value	*p*-Value
(A) Pre-monsoon: bacterial CLPP			
Model		0.06	0.0001*
	Susceptible vs. resistant	0.08	0.0001*
	Susceptible vs. removed	0.02	0.0340
	Resistant vs. removed	0.01	0.1700
	Inter-crown vs. susceptible	0.02	0.1090
	Inter-crown vs. resistant	0.08	0.0001*
	Inter-crown vs. removed	0.03	0.0240
(B) Post-monsoon: bacterial CLPP			
Model		0.03	0.003*
	Susceptible vs. resistant	0.00	0.3150
	Susceptible vs. removed	0.00	0.6290
	Resistant vs. removed	0.00	0.5770
	Inter-crown vs. susceptible	0.04	0.0240
	Inter-crown vs. resistant	0.08	0.0000*
	Inter-crown vs. removed	0.06	0.0050*
(C) Pre-monsoon: Fungal CLPP			
Model		0.04	0.0310*
	Susceptible vs. resistant	0.03	0.0380
	Susceptible vs. removed	0.00	0.6610
	Resistant vs. removed	0.05	0.0010*
	Inter-crown vs. susceptible	0.03	0.0950
	Inter-crown vs. resistant	0.00	0.1950
	Inter-crown vs. removed	0.04	0.0600
(D) Post-monsoon: Fungal CLPP			
Model		0.06	0.0000*
	Susceptible vs. resistant	0.00	0.5360
	Susceptible vs. removed	0.01	0.0540
	Resistant vs. removed	0.02	0.0420
	Inter-crown vs. susceptible	0.09	0.0000*
	Inter-crown vs. resistant	0.08	0.0000*
	Inter-crown vs. removed	0.10	0.0000*

Treatments include soils beneath scale-susceptible, resistant, and scale-removed trees as well as in adjacent inter-crown areas ($n=12$). Significant differences between treatments are indicated by *.

microbial and microarthropod activity and community composition than are litter inputs in this semi-arid ecosystem. Thus, predicting the response of soil microbial communities to herbivory requires understanding how herbivory alters the abiotic environment and how this influence changes with season.

4.1. Does aboveground herbivory alter soil nutrients?

Insect herbivory decreased soil C by 56% and had no effect on soil N, even with a 21% increase in needle litter C inputs and a 56% increase in needle litter N inputs relative to resistant trees (Table 1; Chapman et al., 2003). In contrast to Chapman

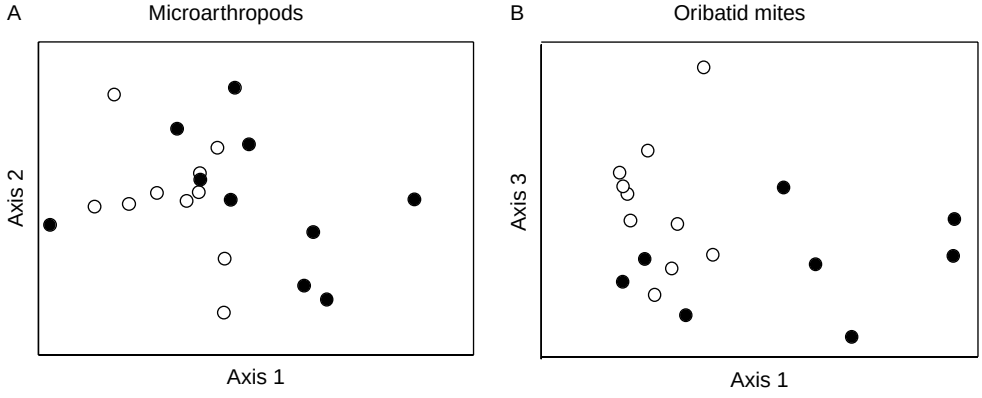

Fig. 3. Microarthropod (A) and oribatid mite (B) communities extracted from susceptible (open circles) and resistant (filled circles) needle litter ($n=10$). Microarthropod and oribatid mite community composition differed significantly in needle litter beneath susceptible and resistant trees. Each point represents the community of microarthropods identified in a litter sample taken beneath an individual tree. Analyses were conducted using multi-response permutation procedures (MRPP).

Table 5
Herbivore effects on litter microarthropod abundance

Litter microarthropods			
Taxa	Trophic group	Resistant	Susceptible
Araneae	Predators	0.3±0.15	nd
Coleoptera	Predators	nd	0.1±0.10
Collembola	Fungivores	nd	3.8±3.05
Diplura	Phytophagous and predators	0.1±0.10	nd
Diptera larvae	Unknown	0.1±0.42	0.6±0.27
Formicidae	Unknown	0.1±0.10	0.2±0.13
Mesostigmata	Predators	0.2±0.13	0.2±0.13
Oribatida	Fungivores and Saprovores	12.3±8.37	20.0±5.62
Prostigmata	Microphytophagus	34.0±16.95	42.6±8.67
Psocoptera	Saprovores and fungivores	0.3±0.15	1.2±0.81
Thysanaptera	Saprovores and fungivores	0.1±0.10	nd

Standard errors are shown beside the means ($n=10$). Not detected is indicated by 'nd'.

et al. (2003); Schuster et al. (2005) found that scale herbivory decreased needle litter production by 25% at this and other southwestern United States sites in two out of three study years. These contrasting effects of herbivory on litter inputs may reflect methodological differences, or more likely, indicate that there is significant interannual variation in herbivory impacts at this site.

4.2. Does aboveground herbivory affect soil and liter microorganisms?

Microbial biomass-N beneath scale susceptible trees was significantly reduced and bacterial CLPPs were distinct relative to resistant trees during the dry, pre-monsoon season, and herbivory reduced enzyme activity during the relatively moist, post-monsoon season (Figs. 1 and 2; Table 4). Scale insect herbivory also caused a shift in the community composition of litter microarthropods (Fig. 3). These patterns may result from herbivore driven changes in substrate quality or soil microclimate. The significant decreases in soil C measured beneath scale-susceptible trees may cause resource limitation of microbial population growth, resulting in greater microbial biomass-N and a distinct bacterial CLPP beneath scale-resistant trees in the pre-monsoon season. Additionally, decreases in C flow as roots, root litter, root exudates, and mycorrhizae beneath scale-susceptible trees (DelVecchio et al., 1993; Gehring et al., 1997; Gehring and Whitham, 1991, 1994, 1995, 2002) may decrease microbial biomass beneath scale-susceptible trees relative to resistant tress. *N*-acetyl-glucosaminidase and β-galactosidase activity rates were also lower beneath susceptible trees relative to resistant trees. *N*-acetyl-glucosaminidase is involved in the mineralization of N from chitin, which might be less abundant beneath the scale-susceptible trees that display significant reductions in mycorrhizal colonization (DelVecchio et al., 1993; Gehring et al., 1997). β-galactosidase, however, is involved in the break down of energy sources and reduced C inputs beneath scale-susceptible trees could result in decreased activity.

Temperature extremes exacerbated by herbivory may also decrease microbial biomass and alter bacterial CLPPs and litter microarthropod community structures beneath scale-susceptible trees during the dry, pre-monsoon season. High summer temperatures, often greater than 40 °C at 5 cm, beneath susceptible trees may directly or indirectly (by increasing evaporation and reducing surface moisture) reduce microbial biomass-N relative to resistant trees. Maximum summer soil temperatures beneath scale-susceptible trees may also favor a more heat-tolerant community of microbes during the hot and dry pre-monsoon sampling period, explaining why this difference goes away during the relatively cool and moist post-monsoon season. Additionally, litter microarthropods respond qualitatively to changes in litter microclimate, particularly in arid land soils, and can be an important link in the decomposer food web (Vossbrinck et al., 1979; Schowalter and Sabin, 1991). If droughts continue to increase in frequency in the southwestern United States, the differences in microbial communities during the dry pre-monsoon season may become more important in shaping nutrient cycling rates over long time periods.

4.3. How does season mediate the effects of herbivores on microorganisms?

Dhillion and Zak (1993) have shown that the structure and function of plant communities in semi-arid regions is highly regulated by the timing of precipitation inputs coupled with N availability. We found that microbial communities also appear to be highly responsive to changes in microclimate and the availability of resources in this semi-arid woodland. Soil microbial biomass was significantly greater during the relatively wet, post-monsoon season and significantly greater beneath tree crowns where there are greater amounts of C to sustain microbial growth. Enzyme activity also responded to seasonal patterns of precipitation. Enzyme rates, fungal CLPPs, and bacterial CLPPs were distinct between the dry and wet seasons (data not shown), a pattern seen at other southwest sites (Boyle et al., 2005). An earlier experiment by Swaty et al. (1998) also found that mycorrhizae at Sunset Crater responded to changes in precipitation and temperature. Their research demonstrated that 70% of the variation in piñon ectomycorrhizal colonization at our site was predicted by a combination of soil moisture and temperature, and that mycorrhizal colonization significantly increased with experimental water additions.

Differences between microbial populations and community structure found in the inter-crown areas and beneath trees also highlights the important role soil microclimate and substrate availability play in structuring microbial communities. Soils in the inter-crown areas had lower N and C concentrations than soils beneath tree crowns (Table 2) and distinct fungal and bacterial CLPPs (Table 4). This result was not surprising as there were few herbaceous plants, few roots, and no accumulated plant litter on the soil surface in the inter-crown areas. Plants provide C in the form of roots, exudates, and litter to soil microbial communities, as well as protection from large temperature fluctuations often found in semi-arid woodlands

(Breshears et al., 1998; Classen et al., 2005). Thus, the differences between organisms found in vegetation free inter-crown areas and beneath tree crowns seem to be driven by increased resources and more moderate soil climates found beneath trees. Furthermore, the study site is in a primary successional state following the volcanic eruption of Sunset Crater, and inter-crown areas likely have been mostly unvegetated during soil development over the past 1000 years. These results support the 'islands of fertility' or 'resource island' hypothesis, where nutrients in semi-arid and arid environments are concentrated beneath plants and inter-crown areas are relatively nutrient poor (Bolton et al., 1993; Smith et al., 1994).

4.4. Do herbivore effects on microorganisms take a long time to develop?

Two lines of evidence suggest that microbial response to aboveground herbivory may take a long time to develop in this semi-arid ecosystem. First, in general, microbial biomass, community structure, and enzyme activity beneath scale-removed trees, while not significant, appears to be intermediate between susceptible and resistant trees, even though scale-removed trees have similar litter inputs (Chapman et al., 2003) and soil microclimate (Classen et al., 2005) as resistant trees. Thus, 17 years of insect removal is apparently insufficient for microbial populations beneath scale-removed trees to become similar to resistant trees. This may be due, in part, to the rate at which processes occur in this ecosystem. Soil N and C stocks change slowly in semi-arid climates where soil processes are limited by precipitation and low above- and below-ground plant litter input (Smith et al., 1994). This lag in the response of nutrient stocks may also alter the rate at which microorganisms respond to herbivore-altered inputs. Second, previous rhizosphere research in this ecosystem revealed significant responses of heterotrophic fungi and ectomycorrhizae to herbivory by a moth insect (*Dioryctria albovittella*) that attacks adult (>60 years) piñon pines (Kuske et al., 2003; Gehring and Whitham, 1991; 1995; Ruel and Whitham, 2002). Kuske et al. (2003) also found that the abundance of decomposers in piñon rhizospheres at our study site was greater beneath 150 year old trees than beneath 60 year old trees as substrates for decomposition, such as tree roots and plant litter, became more abundant. These results suggest that as trees age the response of microbial communities to herbivory may become stronger. Alternatively, our data could be misrepresenting the differences in soil communities by sampling the bulk soil community. These herbivores could have a large impact on microbes and the processes they mediate at small spatial scales, but this effect may be diluted as more of the bulk soil system is sampled.

4.5. Conclusions

Overall, herbivory alters soil and litter microorganisms in this ecosystem, but season strongly influences these effects. These findings suggest that, while nutrient availability may strongly mediate soil properties in mesic ecosystems, it appears to have a much smaller impact in semi-arid ecosystems such as Sunset Crater, especially during drought. Water availability and temperature extremes may be the most important factors affecting microbial activity and community structure in semi-arid ecosystems, while C and nutrient availability are the dominant controllers in more humid ecosystems. If climates in the southwestern US continue to experience prolonged dry periods as a result of climatic change, the microbial community will likely respond in a manner that may reduce ecosystem process rates and slow ecosystem development.

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