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REGULAR ARTICLE

Season mediates herbivore effects on litter and soil microbial abundance and activity in a semi-arid woodland

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Abstract Herbivores can directly impact ecosystem function by altering litter quality of an ecosystem or indirectly by shifting the composition of microbial communities that mediate nutrient processes. We examined the effects of tree susceptibility and resistance to herbivory on litter microarthropod and soil microbial communities to test the general hypothesis that herbivore driven changes in litter inputs and soil microclimate will feedback to the microbial community. Our study population consisted of individual piñon pine trees that were either susceptible or resistant to the stem-boring moth (*Dioryctria albovittella*) and susceptible piñon pine trees from which the moth herbivores have been manually removed since 1982. Moth herbivory increased piñon litter nitrogen concentrations (16%) and decreased canopy precipitation interception (28%), both potentially significant factors influencing litter and soil microbial communities. Our research resulted in three major findings: (1) In spite of an apparent increase in litter quality, herbivory did not change litter microarthropod abundance or species richness. (2) However, susceptibility to herbivores strongly influenced bulk soil microbial communities (i.e., 52% greater abundance beneath herbivore-resistant and herbivore-removal trees than susceptible trees) and alkaline phosphatase activity (i.e., 412% increase beneath susceptible trees relative to other groups). (3) Season had a strong influence on microbial communities (i.e., microbial biomass and alkaline phosphatase activity increased after the summer rains), and their response to herbivore inputs, in this semi-arid ecosystem. Thus, during the dry season plant resistance and susceptibility to a common insect herbivore had little or no observable effects on the belowground organisms and processes we studied, but after the rains, some pronounced effects emerged.

Keywords Community-level physiological profiles · Enzyme activity · Insect herbivory · Microarthropods · Microbial biomass · Semi-arid woodland

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Introduction

Anthropogenic warming of regional climates is predicted to have profound effects on the structure and function of ecosystems (Rustad et al. 2001). Continued increases in atmospheric greenhouse gas concentrations are expected to increase average global surface temperatures by 1.1–6.4 °C (IPCC 2007). Temperature increases are currently modifying the hydrological cycle in both timing and magnitude of precipitation events in ecosystems (Karl and Knight 1998; New et al. 2001). Altered precipitation regimes coupled with increased surface temperatures may increase the occurrence, duration, and intensity of droughts. Increasing drought affects may lead to increased mortality of plants already at their physiological water stress threshold in arid and semi-arid regions. For example, mortality rates for piñon pine in the southwestern United States due to several years of drought (1998-current) is nearly 100% in some areas, while others have escaped the large-scale devastation (Shaw et al. 2005; Breshears et al. 2005). Piñon pine mortality during drought is often associated with insect infestations.

Climatic change will influence the impact of insect herbivores directly by altering their survival, reproduction, dispersal and distribution and indirectly by altering the susceptibility and resistance of their host plants to insect attack (Dale et al. 2001; Bale et al. 2002). Chronic insect herbivory is predicted to increase with drought in semi-arid ecosystems where water-stressed plants are more susceptible to herbivores (e.g., White 1969, 1993; Breshears et al. 2005; Parmesan 2006), raising the question of how herbivory effects may alter ecosystem function. While herbivores are well-known to alter the flow of nutrients in ecosystems (e.g., Ritchie et al. 1998), therefore regulating the microbial communities responsible for nutrient mineralization (e.g., Bardgett 1998), few studies to date have addressed how chronic insect herbivory in a semi-arid ecosystem might alter litter and soil microbial community structure and function (Kuske et al. 2003; Classen et al. 2006). Understanding the response of litter mesofauna and soil microfauna to herbivory in dry and wet seasons may indicate how these populations will respond with increasing droughts and insect outbreaks in semi-arid regions.

We took advantage of a long-term monitoring and removal experiment of the stem-boring moth *Dioryctria albovittella*, from piñon pine, *Pinus edulis* (Brown et al. 2001) to examine how insect herbivory and genetic resistance to herbivory impact soil and litter communities. Differential insect survival rates, long-term presence or absence of these insects on adjacent trees, and allozyme genetic analysis enabled us to categorize the trees in our system as genetically resistant (hereafter ''resistant'' trees) or susceptible (hereafter ''susceptible'' trees) to these herbivores (Mopper et al. 1991; Cobb and Whitham 1993; Gehring et al. 1997; Brown et al. 2001). In addition, moth individuals have been removed from a subgroup of trees since 1982 (hereafter ''removed trees''). Herbivore susceptibility and resistance has pronounced effects on a diverse community of about 1000 species (Whitham et al. 2003) and ecosystem properties, including increasing litter [N] by 16% and decreasing canopy precipitation interception relative to resistant trees (Table 1, Chapman et al. 2003;

Table 1 Herbivore effects on soil temperature (5 cm and 15 cm), soil volumetric water content (VWC, 0–15 cm), canopy precipitation interception, needle and root litter chemistry. Within rows, contrasting letters denote significant differences ($P \leq 0.05$)

Tree Characteristics	Susceptible	Resistant	Removed
Soil microclimate[a]			
Temperature Max 5 cm	21.4[a]	22.4[a]	21.1[a]
Temperature Max 15 cm	19.4[a]	19.1[a]	18.7[a]
VWC 0–15 cm	0.06[a]	0.06[a]	0.06[a]
Canopy interception (%)	33.6[a]	46.6[b]	45.2[b]
Needle chemistry & inputs[b]			
Carbon (%)	47.5[a]	48.8[a]	51.7[a]
Carbon: Nitrogen	82.3[a]	96.2[b]	110.2[c]
Carbon: Phosphorus	542.4[a]	581.1[a]	560.3[a]
Lignin (%)	20.0[a]	19.0[a]	17.2[a]
Lignin: N	23.9[a]	39.5[b]	40.0[ab]
Root chemistry & inputs[c]			
Root inputs (g m^{-2} $year^{-1}$)	47.4[a]	68.9[a]	na
Carbon: Nitrogen	102.6[b]	92.7[a]	na
Phosphorus (%)	0.10[a]	0.10[a]	na
Nitrogen (%)	0.46[b]	0.50[a]	na
Lignin (%)	15.8[a]	15.8[a]	na
Lignin: Nitrogen	34.6[a]	31.6[a]	na

[a] Data published in Classen et al. (2005)

[b] Data published in Chapman et al. (2003)

[c] Data published in Classen et al. (in review)

Classen et al. 2005) and reducing ectomycorrhizal community composition, rhizosphere actinomycetes and heterotrophic fungi (Gehring and Whitham 1991; Gehring et al. 1997; Kuske et al. 2003).

Given these findings, we predicted that aboveground herbivory by the moth insect would alter litter and soil communities in contrasting ways: (1) Herbivore-driven increases in litter quality would increase total litter microarthropod abundance, and orbatid mites in particular and (2) Piñon stress due to moth herbivory would likely decrease soil microbial (bacteria and fungi) biomass and activity. Finally, we predicted that the effects of moth herbivory on soil microbial communities would be more pronounced during the rainy season when microbial activity is at its peak. Answers to these questions are important as they add to the growing body of literature that the interaction of climate change, herbivory, and plant genetics can have major community and ecosystem consequences (e.g., Chapman et al. 2003; Classen et al. 2005).

Materials and methods

Site description and experimental design

Sunset Crater National Monument is situated on the Colorado Plateau in northern Arizona (35 °22′ N, 111 °33′ W). The landscape is covered with a thick layer of cinders and ash due to the eruption of Sunset Crater in 1064 AD (Hooten et al. 2001). Soils are classified as Typic Ustorthents. Sunset Crater is a nutrient-limited, dry piñon-juniper woodland (Cobb et al. 1997; Swaty et al. 1998). Carbonate horizons are not present in the top 60 cm at our site and only show strong reactions to acid from 60 cm to 150 cm depth (G. Newman, unpublished data). Piñon (*Pinus edulis*) and one-seed juniper (*Juniperus monosperma*) are the dominant tree species in the study area. Large, vegetation-free interspaces separate tree canopies at our study sites. 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.

The stem-boring moth (*Dioryctria albovittella*) chronically infests piñon pines of reproductive age (Whitham and Mopper 1985). There are trees at Sunset Crater that are either genetically resistant or susceptible to moth infestation. In addition, moth infestation has been prevented on a select group of reproductively mature trees since 1982 by a single annual treatment with Cygon, a commercial insecticide that has no fertilizer effect (Whitham and Mopper 1985). From each of the three categories (moth-susceptible, naturally moth-resistant, and removal trees), 12 trees were randomly selected from the long-term monitoring and removal study (Brown et al. 2001) for a total of 36 study trees. All trees are growing intermixed at the same site. Previous work at this site has demonstrated that herbivory by the moth insect can significantly alter soil microclimate and needle and root litter characteristics (Table 1, Chapman et al. 2003; Classen et al. 2005). Moth susceptible, moth resistant and moth-removed trees all have similar amounts of total litter production and there are no differences in litterfall timing or input throughout the year (Chapman et al. 2003). Over 80% of annual litterfall occurs between May and August, with 40% during June and July (Chapman et al. 2003).

Under each susceptible, resistant, and removed crown, four mineral soil samples (0–10 cm, approximately 600 g) were taken midway between the trunk and the crown dripline in each cardinal direction. Samples were composited in the lab, sieved to 2 mm, and processed within 48 h. Samples were collected in June prior to summer rains (hereafter ''summer dry season'') and in August during summer rains (hereafter ''summer wet season'') to assess how seasonal changes might alter soil microbial responses to herbivory.

Herbivore effects on litter communities

Litter microarthropods (n = 10) were collected from moth susceptible and moth resistant-tree litter in August of 2002. Four litter samples (O horizon) were collected midway between the trunk and the crown dripline in each of the cardinal directions using a polyvinyl chloride tube (8.89 cm diameter) and hand trowel. Samples were homogenized and emptied into modified Tullgren funnels for 48 h to extract microarthropods (Santos et al. 1978). All microarthropods were counted and identified to morphospecies-level by Karen Lamoncha at Humboldt State University (Camann et al. 2001) and are maintained in a reference collection at Northern Arizona University. Oribatid

mites were further subdivided to the species level. Samples collected from moth-removed trees were not sorted due to cost constraints.

Herbivore effects on soil microbial communities and activity

Microbial biomass-N was determined using a modification of the chloroform fumigation-extraction method described by Haubensak et al. (2002) and analyzed for total N on a Lachat AE Flow Injection Auto-analyzer (Lachat Instruments, Milwaukee WI, USA). Total N in the unfumigated samples was subtracted from total N in the fumigated samples to calculate chloroform labile-N. A KEN correction factor of 0.2 was used to estimate biomass-N (Davidson et al. 1989; Hart et al. 1994).

Microbial activity and function was determined by assaying for eight ecologically relevant enzymes: 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 4-methylumbelliferyl (MUB)-linked substrates: β-D-glucosidase, α-D-glucoside, β-D-galatoside, 7-β-D-xyloside, β-D-cellobioside, *N*-acetyl-β-D-glucosaminide, phosphate disodium salt, and sulfate potassium salt as outlined by Boyle et al. (2005). The first five enzymes assist with the breakdown of energy sources such as carbohydrates and polysaccharides (O'Connell 1987; Eivazi and Tabatabai 1988; Sinsabaugh 1994; Eivazi and Bayan 1996; Boerner et al. 2000). *N*-acetyl-glucosamindase is involved in the mineralization of N from chitin (Olander and Vitousek 2000), phosphatase is involved in the release of inorganic phosphorus (Eivazi and Tabatabai 1977; Bergemeyer 1983; Tarafdar et al. 1989), and sulfatase is involved in the release of inorganic sulfur (Tabatabai and Bremmer 1970; Ganeshamurthy and Neilson 1990; Eivazi and Bayan 1996). Enzyme activities were calculated as μmol product·kg $soil^{-1}$ h^{-1} and then were divided by measured soil C in order to express the values per unit of soil C (μmol product·kg soil C^{-1} h^{-1}).

To assess microbial metabolic activity, we determined community-level physiological profiles (CLPP) using commercially available microtiter plates prepared by Biolog, Inc (Hayward, CA, USA). Biolog is a culture based method, thus it limits our ability to know if these organisms are representative of the organisms at our site, but this method enables us to get a broad index of their potential function. 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). Bacterial plates were read at 590 nm and 750 nm after 72 h and fungal plates were read at 750 nm after 168 h on a MicroMax Plate Reader (Molecular Devices, Sunnyvale, CA, USA). 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. Fungal values were calculated using the 750 nm values (turbidity). Blank well values were subtracted. Data were relativized by plate 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; Garland and Mills 1991; Hungate et al. 2000; Classen et al. 2003). To test for differences in total fungal or bacterial activity, total activity per plate was calculated by summing corrected absorption values for each well.

Herbivore effects on soil properties

Total C concentration, total N concentration, and pH were determined using soil collected during the summer dry season. Total soil C and N concentrations were determined using a Carlo–Erba Model 2500CN elemental analyzer (Milan, Italy). Soil pH was determined using a 1:2 suspension of air-dry soil to 0.01 M $CaCl_2$ solution (McLean 1992; Orion 720A pH meter, Allometrics, Inc., LA, USA). Gravimetric water content (GWC) was determined on soils sampled during the summer-dry and summer-wet seasons by drying (105°C) each sample for

Table 2 Mean (±1 standard error) microarthropod abundance (individuals) in the organic horizon beneath susceptible, resistant, and removed trees. There were no differences detected between treatments ($P \leq 0.10$); dashes (–) indicate that no organisms were found

Litter Microarthropods		Susceptible	Resistant
Taxa	Trophic group		
Araneae	Predators	0.5 ± 0.22	0.4 ± 0.16
Chilopoda	Predators	0.1 ± 0.10	–
Coleoptera	Predators	0.4 ± 0.16	1.0 ± 0.58
Collembola	Fungivores	19.5 ± 15.88	8.0 ± 3.51
Diplura	Phytophagous & Predators	–	0.1 ± 0.10
Diptera larvae	Unknown	2.1 ± 0.96	0.9 ± 0.41
Formicidae	Unknown	0.1 ± 0.10	0.8 ± 0.39
Hemiptera	Phytophagous	–	0.1 ± 0.10
Lepidoptera	Phytophagous	–	0.1 ± 0.10
Mesostigmata	Predators	0.8 ± 0.29	1.2 ± 0.49
Oribatida	Fungivores & Saprovores	40.0 ± 12.36	82.3 ± 31.08
Diplopoda	Detritovores & Herbivores	1.1 ± 0.43	2.0 ± 1.00
Prostigmata	Microphytophagus	52.3 ± 11.51	88.4 ± 25.16
Pseudoscorpion	Predators	0.1 ± 0.10	0.5 ± 0.31
Psocoptera	Saprovores & Fungivores	2.2 ± 1.22	6.1 ± 2.30
Thysanaptera	Saprovores & Fungivores	0.3 ± 0.15	0.6 ± 0.27

2 days. All data are expressed on an oven-dry mass basis.

Statistical analyses

A *t*-test was used to analyze if moth herbivory altered litter microarthropod and orbatid mite abundance and richness. Litter microarthropod communities were also analyzed using multi-response permutation procedures (MRPP) (PC-ORD 4, Mjm Software, Glendale, OR; McCune and Grace 2002). We used one-way analysis of variance tests (ANOVA) to test for differences among susceptible, resistant, and removed soil microbial biomass, enzyme activity, total absorbance of CLPP and soil properties (%C, %N, C:N, pH, GWC). When ANOVA models were significant, a post-hoc LS Means student's t test was conducted to determine differences among treatment means. Where needed, samples were natural log transformed prior to analyses to improve normality and homogeneity of variances; non-transformed data are shown in all tables and figures. Statistics were conducted with JMP 5 statistical software with significance defined as $P \leq 0.10$ (SAS Institute, Cary, NC).

Results

Herbivore effects on litter communities

There were no statistical differences between susceptible and resistant tree litter microarthropod or orbatid mite abundance or richness (Table 2, Fig. 1) or community composition using MRPP (data not shown). Microarthropod functional groups were also not statistically different between trees (Table 2). There was, on average, 156 individual collected per tree ranging from 16 to 608 individuals per tree.

Herbivore effects on soil microbial communities and activity

Moth herbivory had no significant effect on soil microbial biomass-N or soil enzyme activity during the summer dry season (Table 3, Fig. 2) but it did decrease soil fungal CLPPs. Soils beneath moth removed trees had 53% higher fungal CLPP absorbance than susceptible trees, but soils beneath moth-resistant trees were not different from those beneath susceptible or moth-removed trees ($F_{2,30} = 3.25$, $P = 0.05$, Fig. 3). There were no statistical differences

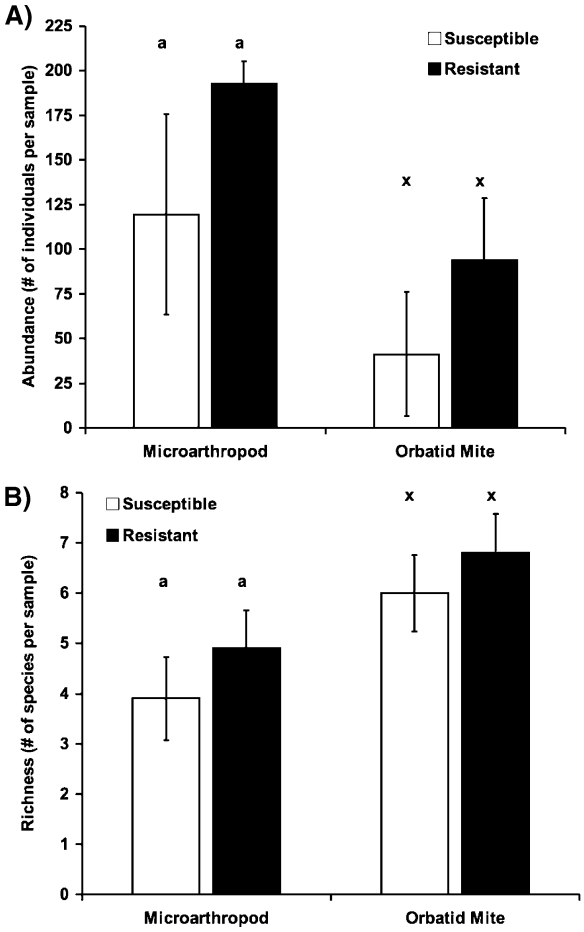

Fig. 1 Litter total microarthropod and orbatid mite abundance (**A**) and richness (**B**) extracted from susceptible and resistant tree litter O-horizon. Letters denote significant differences among treatments within microarthropods or orbatid mites; error bars are ± one SE

in soil bacterial total absorbance among moth susceptible, resistant or removed soils during the summer dry period.

Herbivory had a more pronounced impact on the microbial biomass during the summer wet season. Moth herbivory significantly decreased soil microbial biomass-N relative to moth resistant and removed trees during summer rains ($F_{2,32}$ = 3.62, P < 0.04, Fig. 2). Microbial biomass-N was 52% higher during the summer wet season in both resistant and removed tree soil relative to susceptible tree soil (Fig. 2). Herbivory also significantly altered soil enzyme activity during summer rains. Relative to moth resistant and removed trees, alkaline phosphatase activity was significantly increased 412% beneath moth susceptible trees during summer rains ($F_{2,33}$ = 3.01, P < 0.06,). Moth susceptible soils also had 44% higher fungal total absorbance than removed, but there were no statistically significant differences between resistant and susceptible or resistant and removed tree soil ($F_{2,33}$ = 3.16, P < 0.06, Fig. 3A). Similar to the summer dry season, herbivores had no significant impacts on soil bacterial total absorbance (Fig. 3B).

Overall, summer rains had a large effect on the activity of soil microbes. Microbial biomass during the summer wet season was nearly four-fold greater than during the summer dry season, and this pattern applied across herbivory categories (Fig. 2). Enzyme activity also increased after summer rains (Table 3).

Herbivore effects on soil properties

Moth susceptible, resistant, and removed trees showed no significant differences in soil pH, N concentration, C concentration, and C:N ratios (Table 4). Herbivory also had no impact on soil GWC (Table 4). Relative to the summer dry season, GWC increased in soil during the summer wet season by 387% beneath resistant trees, 477% beneath removed trees and 15% beneath susceptible trees (Table 4).

Discussion

The aim of this study was to determine if insect herbivory alters litter microarthropod diversity and soil microbial community and activity. Previous research in this ecosystem documented that moth herbivory significantly increases litter chemical properties (Table 1, Chapman et al. 2003) and decreases canopy precipitation interception (Table 1, Classen et al. 2005), while reducing ectomycorrhizal colonization and changing ectomycorrhizal community composition (Gehring and Whitham 1991; Gehring and Whitham unpublished data), and reducing rhizosphere actinomycetes and heterotrophic fungi populations (Kuske et al. 2003). Given these findings, we predicted that herbivore-caused increases in litter chemistry and thoughfall would increase litter microarthropod abundances and that tree-stress due to herbivory would decrease soil microbial community functional diversity and activity. Our data show that

Table 3 Mean (±1 standard error) enzyme activities in soils beneath susceptible, resistant, and removed trees, during the summer dry and summer wet seasons. Within season (dry or wet) and rows, contrasting letters denote significant differences ($P < 0.10$)

Enzyme (μmol kg^{-1} soil C h^{-1})	Summer dry season			Summer wet season		
	Susceptible	Resistant	Removed	Susceptible	Resistant	Removed
Ligno-celluloytic enzyme activities						
α-1,4-glucosidase	49.26 [a] (36.19)	202.71 [a] (74.49)	189.63 [a] (89.03)	926.91 [a] (487.74)	302.53 [a] (153.68)	112.86 [a] (52.65)
β-1,4-glucosidase	239.32 [a] (88.49)	592.14 [a] (180.79)	716.81 [a] (221.22)	4324.66 [a] (1852.16)	1586.61 [a] (612.74)	890.64 [a] (303.43)
Cellobiohydrolase	0.00 [a] (0.00)	30.01 [a] (20.51)	101.40 [a] (52.07)	669.07 [a] (323.02)	223.62 [a] (144.49)	104.28 [a] (57.82)
β-galactosidase	15.06 [a] (14.02)	83.24 [a] (44.12)	99.40 [a] (68.71)	1011.60 [a] (514.44)	270.79 [a] (186.44)	125.46 [a] (66.86)
β-xylosidase	17.44 [a] (15.88)	88.85 [a] (41.50)	108.18 [a] (58.06)	891.23 [a] (490.23)	264.18 [a] (154.49)	453.70 [a] (318.72)
Nutrient mineralizing enzyme activities						
N-acetyl-glucosaminidase	13.85 [a] (10.10)	113.69 [a] (50.30)	289.09 [a] (115.85)	1614.36 [a] (686.01)	1017.02 [a] (439.90)	419.89 [a] (191.52)
Alkaline phosphatase	52.21 [a] (29.39)	469.04 [a] (364.87)	618.28 [a] (248.07)	8760.37 [a] (3304.57)	3172.38[b] (1407.67)	1710.39 [b] (804.94)
Sulfatase	0.00 [a] (0.00)	0.00 [a] (0.00)	21.46 [a] (15.15)	177.22 [a] (103.28)	82.25 [a] (55.51)	13.02 [a] (13.02)

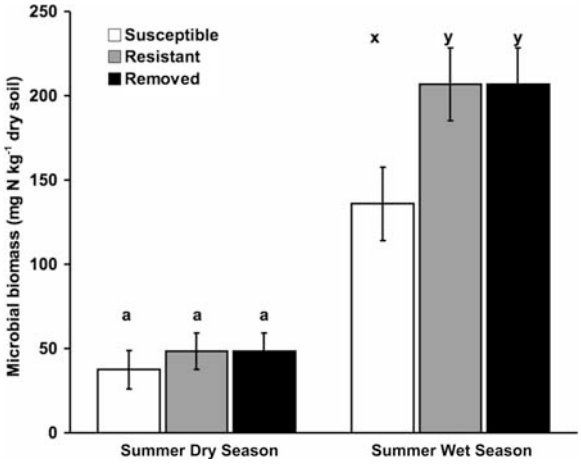

Fig. 2 Mean microbial biomass-N in mineral soil (0–10 cm) sampled beneath susceptible, resistant, and removed trees in the summer dry and summer wet seasons. Contrasting letters within sampling dates denote significant differences among treatments ($P \leq 0.10$); error bars are ± 1 SE

insect herbivores can regulate soil microbial populations, but seasonal dynamics (even during protracted drought) can exert strong control over the effect that herbivores have on microbial abundance and activity. Specifically, our work resulted in three major conclusions: (1) In spite of an increase in litter quality, herbivory does not appear to change litter microarthropod abundance or species richness. (2) Herbivore susceptibility alters soil microbial communities, but not soil properties. (3) Season has a strong influence on how aboveground insect herbivory mediates the soil microbial community in this semi-arid ecosystem. These findings will be discussed in turn.

Moth herbivory increased litter nitrogen inputs by 16% and decreased canopy precipitation interception – both factors that can increase litter microarthropod abundance (Hansen and Coleman 1998; Hansen 1999; Reynolds et al. 2003). Work in other ecosystems found that abundance of litter and soil microarthropods responded positively to herbivory (Seastedt and Crossly 1983; Schowalter and Sabin 1991; Reynolds et al. 2003), but these responses were likely due to an increase in frass inputs or thoughfall chemistry quality, and moth herbivory in this ecosystem has no effect on either of these factors (AT Classen and SK Chapman, unpublished data). Abundance of microarthropods found at this site was very low, even when compared to microarthropod

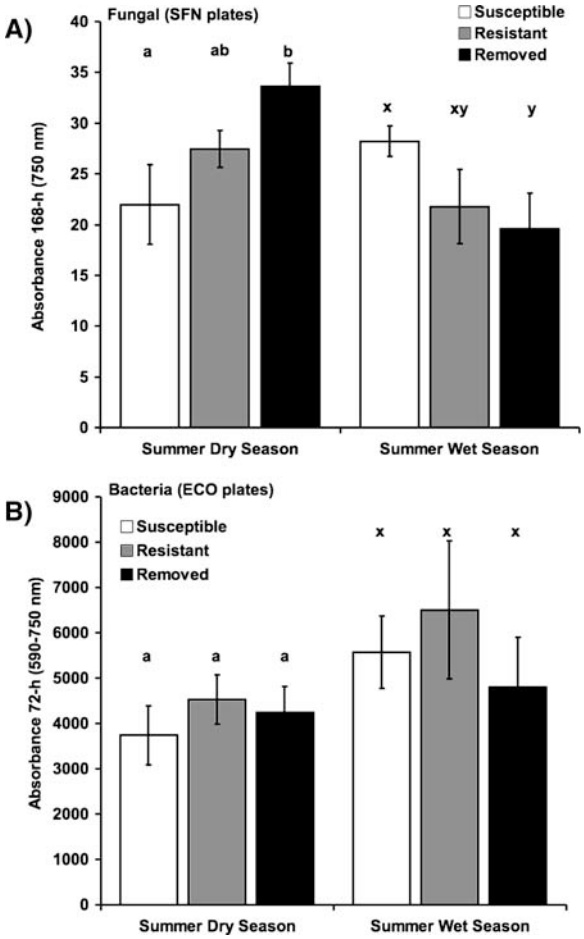

Fig. 3 Fungal (**A**) and bacterial (**B**) community-level physiological profile (CLPP) total well absorbance in mineral soil (0–10 cm) sampled beneath susceptible, resistant, and removed trees during the summer dry and summer wet seasons. Contrasting letters within sampling dates denote significant differences among treatments ($P \leq 0.10$); error bars are ±1 SE

numbers found in other dry land ecosystems (Whitford 1996). In addition, we measured microarthropod abundances before summer rains when the organisms were likely water limited. Microarthropods respond strongly to season and water availability (Schowalter and Sabin 1991; Reynolds et al. 2003; Osler et al. 2004; Taylor and Wolters 2005), and so it is possible there could be more of a response during the summer rainy season when microarthropod activity and abundance might be greater.

Insect herbivores in this ecosystem reduce cone and seed production (Cobb et al. 2002; Mueller et al. 2005), tree growth (Whitham and Mopper 1985; Ruel and Whitham 2002), and mycorrhizal associations (Gehring and Whitham 1991, 1997), all indications of tree stress and all factors that may alter soil properties through time. We predicted that herbivory would decrease bulk soil CLPPs and enzyme activity, and microbial biomass and indeed this was the case. Herbivory decreased microbial biomass, which was 52% higher in resistant and removed soil relative to susceptible soil during the summer wet season. These results are similar to Kuske et al. (2003) who found that actinomycetes and heterotrophic fungi abundance were lower in the rhizosphere of moth-infested piñon. However, alkaline phosphatase, an enzyme involved in the release of inorganic phosphorus, was 412% higher beneath susceptible trees during the summer wet season, indicating an increase in activity relative to resistant and removed trees. This may be in response to lower P availability caused by a reduction in mycorrhizal infection on susceptible trees (Gehring and Whitham 1991, 1997). Fungal metabolic activity, assed using CLPPs, was also

Table 4 Mean (±1 standard error) soil pH, total carbon concentration (C), total nitrogen concentration (N), C:N mass ratio, bulk density, and gravimetric water content (GWC) in the mineral soil (0–10 cm) of susceptible, resistant, and removed trees. There were no differences detected among treatments ($P \leq 0.10$)

Soil Chemistry	Susceptible	Resistant	Removed
pH	6.6 ± 0.15	6.8 ± 0.11	6.7 ± 0.13
N (g kg^{-1})	10.0 ± 0.2	20.0 ± 0.3	20.0 ± 0.3
C (g kg^{-1})	21.0 ± 3.5	28.0 ± 5.4	24.0 ± 4.6
C:N	16.5 ± 0.56	16.0 ± 0.66	16.2 ± 0.62
Bulk density (mg m^{-3})[a]	1.16 ± 0.03	1.12 ± 0.02	
GWC summer dry season (%)	6.2 ± 3.1	1.6 ± 0.4	1.7 ± 0.5
GWC summer wet season (%)	7.1 ± 0.8	7.8 ± 0.9	9.4 ± 1.3

[a] Unpublished data (AT Classen). No data are available for the resistant trees

increased beneath susceptible trees during the wet season. Previous research has shown that negative correlations between enzyme activity and nutrient availability are often present when nutrients are limiting because the activity of the enzyme may be regulated by the supply of the nutrient (Olander and Vitousek 2000). Physiological stress due to herbivory may limit nutrient input to the soil system via decreases in root exudation and production. Therefore, nutrient limitation may increase activity by certain members of the soil community, while decreasing overall soil community biomass (Kuske et al. 2003).

We predicted that insect herbivory would influence soil properties by increasing litter input quality ([N]) and by possibly decreasing C allocation belowground (stress response). Surprisingly, herbivory had no detectable effect on total soil bulk density, N or C concentration. The trees in our study have been insect resistant or susceptible for 60 years to 120 years, thus we predicted a change in soil nutrient stocks, if there was one, would be detected. The soils at Sunset Crater are nutrient poor, unstructured, and poorly developed. These qualities may make it difficult to detect small changes in nutrient stocks over time. Additionally, because this is a semi-arid ecosystem, decomposition rates and nutrient turnover is slow, especially during an ongoing drought, thus carbon movement into the soil profile may be happening over a long time scale (Chapman et al. 2003).

Our results are characteristic of plant genotype x environment interactions, which are increasingly recognized as being key to understanding the community and ecosystem-level consequences of heritable variation in plant traits, such as herbivore resistance at Sunset Crater (e.g., Shuster et al. 2006; Bailey et al. 2006; Whitham et al. 2006). The seasonal response of micro-organisms in this ecosystem appears to reflect the seasonal abundance of precipitation at our site, where the dry pre-summer rain environment limits microbial activity. Previous work in this and other semi-arid piñon-juniper ecosystems have found that enzyme activity and microbial biomass was higher during the wet season (Krämer and Green 2000; Classen et al. 2006). Our results indicate that, in this semi-arid ecosystem, season has a stronger influence than herbivore resistance or tolerance in host-trees on soil microbial communities and activity. Our results come from one of the longest-running herbivore exclusion experiments and stress the importance of including temporal dynamics when trying to understand the links between above- and below-ground communities, and under what environmental conditions we can expect these links to be strong or weak.

In conclusion, our results suggest that microbial populations in semi-arid ecosystems under drought conditions may respond differently to insect herbivory than populations in more mesic ecosystems where water is less limiting to activity. Understanding the interactions among water availability, substrate quality, herbivory, and microbial activity remains an important research priority if we are to better predict how herbivory will shape semi-arid ecosystems in the future. Insect herbivores are predicted to readily respond to climatic changes, especially in dry land ecosystems, thus understanding the degree to which insect herbivory may shape ecosystem processes is an increasingly important global management issue.

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