5-2013

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Recommended Citation

Aimee T. Classen, Samantha K. Chapman, Thomas G. Whitham, Stephen C. Hart, and George W. Koch 2013. Long-term insect herbivory slows soil development in an arid ecosystem. Ecosphere 4:art52. http://dx.doi.org/10.1890/ES12-00411.1

esa

ECOSPHERE

Long-term insect herbivory slows soil development in an arid ecosystem

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Citation: Classen, A. T., S. K. Chapman, T. G. Whitham, S. C. Hart, and G. W. Koch. 2013. Long-term insect herbivory slows soil development in an arid ecosystem. Ecosphere 4(5):52. http://dx.doi.org/10.1890/ES12-00411.1

Abstract. Although herbivores are well known to alter litter inputs and soil nutrient fluxes, their long-term influences on soil development are largely unknown because of the difficulty of detecting and attributing changes in carbon and nutrient pools against large background levels. The early phase of primary succession reduces this signal-to-noise problem, particularly in arid systems where individual plants can form islands of fertility. We used natural variation in tree-resistance to herbivory, and a 15 year herbivore-removal experiment in an Arizona piñon-juniper woodland that was established on cinder soils following a volcanic eruption, to quantify how herbivory shapes the development of soil carbon (C) and nitrogen (N) over 36–54 years (i.e., the ages of the trees used in our study). In this semi-arid ecosystem, trees are widely spaced on the landscape, which allows direct examination of herbivore impacts on the nutrient-poor cinder soils. Although chronic insect herbivory increased annual litterfall N per unit area by 50% in this woodland, it slowed annual tree-level soil C and N accumulation by 111% and 96%, respectively. Despite the reduction in soil C accumulation, short-term litterfall-C inputs and soil C-efflux rates per unit soil surface were not impacted by herbivory. Our results demonstrate that the effects of herbivores on soil C and N *fluxes* and soil C and N *accumulation* are not necessarily congruent: herbivores can increase N in litterfall, but over time their impact on plant growth and development can slow soil development. In sum, because herbivores slow tree growth, they slow soil development on the landscape.

Key words: carbon cycling; insect herbivory; nitrogen cycling; pine; piñon-juniper woodland; primary succession; soil development.

Received 26 December 2012; revised 25 February 2013; accepted 26 February 2013; final version received 2 April 2013; **published** 2 May 2013. Corresponding Editor: S. L. Collins.

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INTRODUCTION

Insect herbivores can have variable impacts on nutrient cycling in ecosystems; they can increase (e.g., Chapman et al. 2003, Frost and Hunter 2004, Fonte and Schowalter 2005), slow (e.g., Hartley and Jones 2003), have mixed, or no impact on nutrient cycling (e.g., Classen et al. 2007*a*). This variation in response likely reflects the interaction of diverse herbivore traits with the properties of contrasting ecosystems (e.g., outbreak vs. chronic herbivory, mesic vs. arid ecosystems). Hunter (2001) described multiple pathways by which insect herbivores could alter

nutrient cycling including the addition of insect waste products and bodies, altered canopy leachates and litter chemistry, and herbivore-driven shifts in plant and soil communities. While many recent studies have documented the impacts of insect herbivory on ecosystems (e.g., Belovsky and Slade 2000, Frost and Hunter 2004, Madritch et al. 2007, Blue et al. 2011, Schowalter et al. 2011, Zhang et al. 2011), it is difficult to assess insect herbivore impacts on soil C and N accumulation in mesic ecosystem soils because the canopies of plants overlap, background pool sizes are high relative to changes in input rates associated with herbivory, and herbivore manipulation experiments may not be sufficiently long to detect a signal. Thus, it remains unclear whether non-outbreak insect herbivores influence larger-scale, longer-term processes such as the development of soil total carbon (C) and nitrogen (N) stocks.

Primary production in arid, nutrient-poor ecosystems is low and trees can create "islands of fertility" on the landscape, in which an isolated, individual tree has a singular impact on the soil beneath it (Schlesinger et al. 1996; Fig. 1). Thus, small changes in productivity or resource allocation over time in these ecosystems can impact, in measurable ways, the soil "footprint" of plants and thus total C and N pools and accumulation at the landscape scale (Neff et al. 2009, Reiley et al. 2010). Such is the case in the early successional piñon-juniper woodland at Sunset Crater National Monument in northern Arizona, USA. Here the young volcanic cinder soils (Sunset Crater erupted c. 1064) have small C and N pools (Classen et al. 2006), the piñons (*Pinus edulis*) present are likely the among the first plant colonists, N deposition to the system is low (Classen, *unpublished data*), and the widely-spaced trees anchor individual plant-soil islands. Individual piñons that are either susceptible or resistant to herbivores form a mosaic of phenotypes on the landscape (Fig. 1). Herbivory on susceptible juvenile piñons by the mesophyll-feeding scale insect (*Matsucoccus acalyptus*) is chronic and causes chlorosis and early needle abscission, resulting in only one to two years of foliage rather than the usual six to eight years present on scale-resistant trees (Cobb and Whitham 1993; see Fig. 1). Scale insect herbivory reduces leaf biomass by up to 75%, slowing growth and resulting in smaller trees for a given age (Trotter et al. 2002). These changes in primary productivity may yield different soil C and N accumulation rates beneath each tree in these "islands of fertility."

A long-term herbivore removal experiment at Sunset Crater has clearly documented the community and ecosystem impacts of herbivory (e.g., Gehring et al. 1997), including changes in litter chemical quality, soil microclimate, litter decomposition, and other components of ecosystem function (e.g., Chapman et al. 2003, Classen et al. 2005, Schuster et al. 2005, Classen et al. 2007*a*). Perhaps the most striking of these results is that scale-susceptible trees produce twice the N inputs per unit soil area as resistant trees, primarily due to herbivore-increases in litter N concentrations caused by abbreviated resorption (Chapman et al. 2003). Because herbivory substantially increases needle litter N inputs, we hypothesized that soils beneath susceptible trees would have increased rates of N mineralization compared to the herbivore-resistant and herbivore-removal trees. Due their slower growth, and the likely dominance of soil CO_2-efflux by root respiration, we hypothesized that herbivore-susceptible trees would have lower soil CO_2-efflux rates per unit ground area than herbivore-resistant and herbivore-removal trees. We hypothesized that the short-term changes in fluxes measured over two years would be manifested in long-term changes in soil C and N pools over the 36 to 54 year life span of trees used in our study. Lastly, we hypothesized that herbivory would decrease C accumulation and increase N accumulation in soils beneath susceptible relative to resistant and removed trees. Owing to the isolation of individual trees in this ecosystem, it is possible to detect the influence of individual trees on soil C and N content as well as their whole contribution to ecosystem C and N dynamics.

Methods

We conducted this study in 5 ha of piñon-juniper woodland located adjacent to Sunset Crater National Monument (35°22′ N, 111°33′ W), northeast of Flagstaff, Arizona, USA, on the Colorado Plateau. Sunset Crater erupted in 1064 AD and covered the landscape with a thick layer

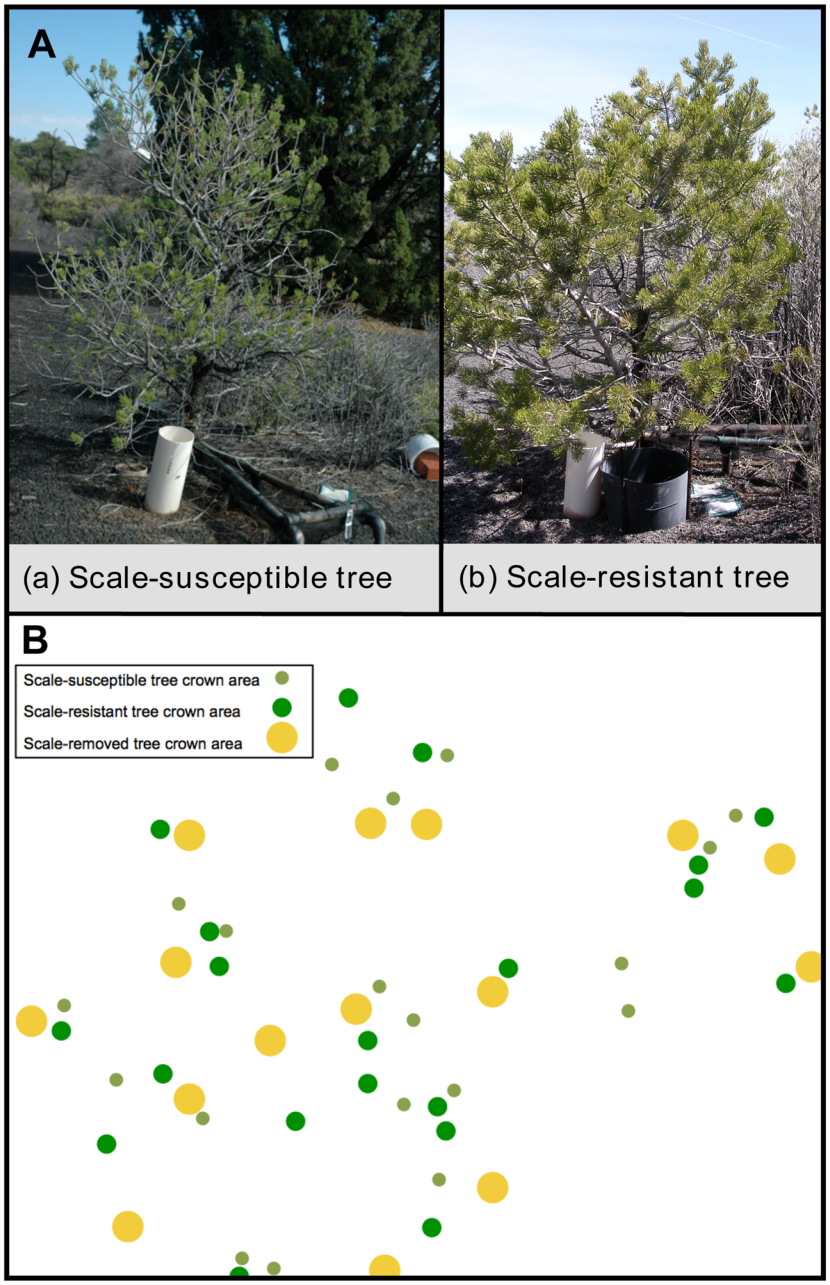

Fig. 1. (A) Scale herbivory had a striking impact on piñon crown architecture in this ecosystem. Scale-susceptible trees had a 'poodle-tail' architecture with only 1–2 years of needles (a), while scale resistant trees had a full complement of 7-years of needles (b). For spatial scale, note the needle litter decomposition bags (10 × 10 cm) on the ground beneath tree crowns and the white throughfall collector that is the same height and diameter as a 1-L Nalgene bottle. (B) A map schematic of projected crown areas showing the distribution and relative sizes of individual susceptible, resistant, and removed piñons on a small subsection of the field site. Note that susceptible tree areas (pale green dots) were smaller than resistant tree areas (dark green dots), and the removed trees had the largest areas (yellow dots) suggesting a possible growth cost to being insect-resistant. The trees had a patchy distribution on the landscape with large, intercrown areas.

of ash and cinders denuding the area of vegetation (Hooten et al. 2001). The soils at this site are classified in the Soil Taxonomic Family as cindery, mesic Typic Ustorthents and soil pH beneath piñons (0–30 cm) averages 6.9 (Classen et al. 2006, Classen et al. 2007*b*). The soils at this site are very coarse textured and range between 83-98% sand, 1–14% silt, and 0–8% clay; with no differences in texture among our treatment trees (sample size n = 20). The elevation at our site is approximately 2100 m and thirty-year means of precipitation and air temperature are 432 mm and 8.6°C, respectively (Selmants and Hart 2010). The dominant woody species is piñon and other woody species include: one-seeded juniper (*Juniperus monosperma*), Mormon tea (*Ephedra viridis*), Apache plume (*Fallugia paradoxa*), and squawbush (*Rhus trilobata*). Piñons and other trees and shrubs at this site are widely spaced (~2–10 m apart), with large, vegetation free, inter-tree areas. Nitrogen inputs due to wet deposition are low at our site approximately 1 kg h^{-1} yr^{-1} (local NADP data). Similarly, growth of piñon increases in this ecosystem when N fertilizer and water are added indicating that piñons are both water and nutrient limited (Looney et al. 2012).

The experimental design for this study is detailed elsewhere (Chapman et al. 2003, Classen et al. 2005). Briefly, herbivory by the piñon needle-scale on piñons has been monitored for over 25 years. Scale herbivory phenotypes were determined in 1985 (Cobb and Whitham 1993). Scale-susceptible trees are characterized by high numbers of scales on needles as well as the presence of only two age-cohorts of needles, the older of which is chlorotic and ready to abscise. Scale-susceptible litter has a 50% higher N concentration than scale-resistant litter, but contains similar amounts of lignin and secondary chemicals (Chapman et al. 2003). Scale-resistant trees have few or no scales and five to seven age-cohorts of green needles. Using scale transfer experiments, the susceptibility or resistance of individual trees was confirmed by scale mortality on resistant trees being 3.4 times that on susceptible tree (68% vs. 20%, respectively; Cobb and Whitham 1993). At the time of this study, a subset of susceptible trees had been maintained free of scale insects for a minimum of 15 years by removing the scale egg clusters from the base of the tree prior to insect emergence (see Gehring et al. 1997). These "scale-removed" trees have recovered to resemble resistant phenotypes, demonstrating that scale insects are responsible for the observed changes in tree architecture and litter properties. Twenty individual trees from each of the three herbivore conditions (scale susceptible, scale removed, and scale resistant; 60 trees total; n = 20) were randomly selected to measure nutrient pools and fluxes; trees were chosen to be at least two tree crown widths from their nearest neighbor, the distance of maximum root extension by piñons at this site. To decrease disturbance to these long-term study trees (both collecting soils and disturbing the crown), we tried to use the same soil core for several measurements (e.g., total N and N-min). Additionally, while we recognize that modeled data indicate that C and N pools can vary at different locations beneath tree crowns in semi-arid systems, to sample at numerous crown locations in our study would have killed our trees and is beyond the scope of the questions we asked (Throop and Archer 2008).

Annual litterfall C and N

Annual litterfall and C and N litter inputs were estimated based on litter collections that occurred from 2000 to 2001, which are extensively described in Chapman et al. (2003). Briefly, we placed isosceles triangle-shaped litter traps (individually constructed based on the crown area of each individual) lined with one-mm nylon mesh beneath each tree. Each trap was placed in a random compass direction under each of the 60 trees and sampled 1/20 (18°) of the projected crown area. Litter was collected in April, June, July, and November of 2000 and March of 2001 to coincide with times of peak litterfall. Litter collected in traps was sorted (to remove non-needle material), dried at 70°C, weighed, and subsampled for chemical analyses. Litter subsamples were ground, digested, and analyzed for TKN on a Lachat flow injection (Lachat Instruments, CO, USA). Carbon was analyzed with a Carlo-Erba Model 2500CN elemental analyzer (Milan, Italy). Litter nitrogen inputs were calculated by multiplying litter N-concentration by litterfall rates (g m^{-2} yr^{-1}). Scales make up a maximum of ~2% of litterfall (this includes frass and excrement), thus labile C and N inputs from the scale insects in this ecosystem is low and

would not contribute to differences among our treatments (Classen et al. 2007*a*).

Soil net N-mineralization and carbon dioxide efflux

Net N-mineralization rates and net nitrification rates were measured in situ in the mineral soil over concurrent six-month long incubation periods using the resin-core method. Here, we focus on mineral soil because in a companion study we discussed litter nutrient turnovers during litter decomposition (Classen et al. 2007*a*). Over two years, we sampled net N mineralization in four 6-month measuring periods (Hart and Firestone 1989, Hart et al. 1994, Robertson et al. 1999). From 2000 to 2002, paired soil cores (7 cm diameter, 0–30 cm depth; the entire active rooting depth of our trees) were taken beneath the susceptible, resistant, and removed trees at the onset of two major changes in soil climate that occur in these ecosystems: October–April (cool season) and April–October (warm season). Sampling locations beneath a given tree were determined at random and, when present, the litter layer was removed. One set of cores was removed at each sampling period to avoid oversampling the long-term experimental trees (Classen et al. 2007*b*); over two years, 8 cores were collected to assess C and N dynamics. Upon installation in the field, one soil core from each pair was returned to the laboratory for gravimetric water content and inorganic N analyses. The other soil core was left in the field to incubate open in a PVC pipe with an ion exchange resin bag attached to the bottom to collect inorganic N leached from the core (Hart et al. 1994, Robertson et al. 1999). Because N deposition rates in this ecosystem are low and the ecosystem is very dry, we did not install resin bags on the top of the cores. Upon removal from the field, all soils were sieved to 4 mm and resins were rinsed with DI water. Soils in this ecosystem are very coarse textured containing between 83 and 98% sand sized particles, thus a 4 mm sieve size was used to remove coarse fragments. Soils and resins were extracted with 2M KCl and then analyzed for NH_4^+ and NO_3^- using a Lachat Flow Injection Analyzer (Lachat Instruments, CO, USA; Robertson et al. 1999). The difference in inorganic N pools in the incubated soil core and inorganic N collected on the resin bag minus initial soil pools were used to estimate rates of soil net N-transformations over the incubation period (Hart et al. 1994, Robertson et al. 1999). Annual N-mineralization rate estimates were determined by adding the seasonal rates. Bulk density was calculated for each tree as well as for the inter-crown areas (Classen et al. 2007*b*).

We measured carbon dioxide (CO_2) efflux in situ every two weeks during the growing season of 2000 and 2001 beneath susceptible, resistant, and removed trees using the soda lime static chamber technique (Edwards 1982, Grogan 1998). This method enabled 24-h integrated measurements on a large number of samples (60) almost simultaneously (Hutchinson and Rochette 2003). Work published from a local ponderosa pine (*Pinus ponderosa*) site found that soda lime measurements were significantly correlated with measurements made by a dynamic chamber infrared gas analyzer (Kaye and Hart 1998).

Soil potential N-mineralization and microbial C-efflux

To distinguish between the separate and interacting influences of herbivore-induced changes in litterfall quality and those due to changes in soil temperature and moisture, we conducted a 64-d soil laboratory-incubation using standard soil methods (Robertson et al. 1999). Soils removed from the initial cores collected in the field experiment in April 2002 were used in this incubation experiment.

Soil samples were sieved (4-mm), moisture was adjusted to reach field capacity, and three 20 mL subsamples were placed into glass sample vials. One sample was extracted immediately with 2M KCl to determine initial inorganic-N pool sizes while the other two subsamples were incubated in wide-mouth glass 1-quart Mason jars at lab temperatures for 31 and 64 days, respectively, and then extracted with 2M KCL. In addition to the soil sample cups, each incubation jar contained a small vial of water to help maintain humidity. Soil extracts were analyzed for NH_4^+ and NO_3^- using a Lachat Flow Injection Analyzer (Lachat Instruments, CO, USA). The difference between inorganic N pools in the incubated soil core minus initial soil pools were used to estimate rates of potential soil net N transformations over the incubation period.

Soil C-efflux was measured on the incubations above using standard methods (Robertson et al. 1999). Briefly, CO_2 samples were extracted from the headspace of the jars with a needle and syringe through septa fitted in each incubation jar and analyzed using a gas chromatograph (fitted with a thermal conductivity detector) after day 3, 10, 17, 31, 42, and 64. Incubation jars were flushed for two minutes with ambient air after each collection. Total CO_2 evolved after 64 days was calculated by adding the CO_2-C evolved at each of the sampling dates.

Soil total C and N pools and accumulation rates

Total soil C and N were measured in November of 2000 (20 susceptible, 20 resistant, and 20 removed trees; $n = 20$). After taking the 30 cm cores, soils were returned to the lab, sieved to 4-mm, air-dried, ground to a fine powder using a mortar and pestle, and analyzed for total C and N on a Carlo-Erba Model 2500CN elemental analyzer (Milan, Italy).

To estimate rates of soil total C and N accumulation, we measured the age of trees overlying each soil sample using standard dendrochronological techniques (Stokes and Smiley 1996). Trees cores were extracted with a 4-mm Mora increment borer, mounted, and sanded with a 120 grit-belt sander then by hand with 400, 500, and 1500 grit sandpaper. Tree ages were determined by counting annual rings viewed through a dendrochronology microscope (Velmex, Bloomfield, NY; see Trotter et al. 2002).

We calculated average C and N pools (g m^{-2}), accumulation (g m^{-2} yr^{-1}), as well as tree-level accumulation (g $tree^{-1}$ yr^{-1}). Soil C and N pools were determined by multiplying soil C and N concentrations by bulk density of each individual soil core to 30 cm. Soil C and N accumulation rates were calculated by dividing the soil pools (g m^{-2}) by the age of the tree under which the soil was sampled (g m^{-2} yr^{-1}). Tree-level soil nutrient accumulation rates were determined in order to provide an examination of the "footprint" of herbivory on the landscape and were calculated from nutrient pools, tree age, and total crown area:

$$[((\text{g of nutrient})/\text{m}^2 \times \pi r^2)/\text{tree age}].$$

Crown area (m^2) was calculated as πr^2, where r was the average radius based on north–south and east–west crown diameter measurements. The total nutrient accumulation was calculated on an individual tree basis where the average total value of C or N in the soil core on (g m^{-2}) over the 0–30 cm depth was multiplied by the total tree crown area. Implicitly, this calculation assumed that soil C and N accumulation in these young soils was approximately linear and there were very small total C and N pools in the soil prior to tree establishment. There were two lines of evidence that supported these assumptions: (1) C and N pools in inter-tree areas were very low (see values below) and were likely higher than the sites where trees established 50–75 years ago; and (2) this was a primary successional ecosystem. There was very little vegetation across the landscape (Fig. 1). Finally, litter decomposition and nutrient release proceeded lineally in this ecosystem over two years consistent with the assumption that C and N accumulation in mineral soil were also linear (Classen et al. 2007*a*).

Susceptible, resistant, and removed trees varied in age because at the beginning of this experiment (25 years ago), susceptible, resistant, and (soon to be) removed trees were paired for size without knowledge of individual tree age. Susceptible trees grew more slowly than resistant trees and thus susceptible trees were older than resistant trees when they were initially paired for size. Susceptible trees averaged 54 ± 3 yr, scale-resistant trees averaged 36 ± 4 yr, and scale removed-trees average 48 ± 4 yr ($F = 7.31$, $P < 0.002$). To account for the difference in tree age, the development of C and N pools were expressed as annual rates. In order to make our data more comparable to other studies, we also calculated beneath-tree pools of C and N (g m^{-2}) and accumulation rates of C and N on a landscape scale as g m^{-2} yr^{-1}.

Statistics

For all statistical analyses, we used JMP 8 statistical package with significance defined as $P < 0.05$ (SAS Institute 2001; Pacific Grove, CA). Full-factorial fixed-effects analyses of variance (ANOVA) were conducted to distinguish among susceptible, resistant, and removed trees (inputs and fluxes) and susceptible, resistant, and removed trees (long-term pools and accumulation).

When data violated the assumptions of ANOVA, they were log or arc-sign transformed. Repeated measures ANOVA were used to analyze time series data. Post-hoc Tukey's tests were conducted to distinguish pair-wise comparisons of susceptible, resistant, and removed tree impacts on soil nutrient pools and accumulation.

Results

Herbivore influences on soil C inputs and fluxes

Needle litterfall biomass, on a per area basis, did not differ among scale-susceptible, resistant, and removed trees ($F = 1.92$, $P = 0.16$; Fig. 2A). There were also no statistical differences in growing season soil C-efflux among susceptible, resistant, and removed trees ($F = 0.02$, $P = 0.63$; Fig. 2C); however, soil C-efflux did vary between years ($F = 1.88$, $P < 0.0001$; Fig. 2C). Similarly, C-efflux after 64 days of laboratory incubation was not different among susceptible, resistant, and removed trees ($F = 0.01$, $P = 0.99$; Fig. 2E); however, in this case, C-efflux also did not differ over time (data not shown).

Herbivore influences on soil N inputs and fluxes

Scale herbivory caused litter N inputs (g m^{-2} yr^{-1}) beneath scale-susceptible trees to be nearly double that of inputs beneath scale-resistant and scale-removed trees ($F = 12.19$, $P < 0.0001$; Fig. 2B). Additionally, scale herbivory increased litter N concentration approximately 50% relative to resistant and removed trees. Litter carbon to nitrogen (C:N) values were lowest for susceptible trees (64 ± 5), and similar between resistant (87 ± 4) and removed (88 ± 4.7) trees ($F = 8.66$, $P = 0.001$; data not shown). Interestingly, there were no statistical differences in net N-mineralization among susceptible, resistant, and removed trees ($F = 1.08$, $P = 0.36$, Fig. 2D) and years ($F = 1.83$, $P = 0.18$), nor were there any year × treatment interactions ($F = 0.78$, $P = 0.51$). There were also no differences in net nitrification rates among susceptible, resistant, or removed trees or in the total inorganic N extracted from the incubated resin bags (data not shown). Similarly, net N mineralization measured under laboratory conditions did not differ over 31 days ($F = 1.54$, $P = 0.22$) or 64 days of incubation ($F = 1.54$, $P = 0.22$) or 64 days of incubation ($F = 1.74$, $P = 0.19$, Fig. 2F). We were unable to detect differences in laboratory rates of net nitrification or in field extractable NH_4^+ or NO_3^- (data not shown). For simplicity, data presented in Fig. 1F are laboratory-based rates of soil net N mineralization over 64 days because there were no differences in laboratory-based net N mineralization or nitrification after 31 days, and the patterns were consistent across time.

Soil C and N pools and accumulation

Scale susceptible, resistant, and removed trees did not differ in soil C (g m^{-2}) pools ($F = 0.55$, $P = 0.577$; Fig. 3A) or soil N (g m^{-2}) pools ($F = 1.13$, $P = 0.331$; Fig. 3B). However, they did differ significantly in accumulation at both the landscape and tree-level. Scale resistant trees had 152% higher soil C accumulation (g m^{-2} yr^{-1}) than susceptible trees and 110% higher soil C accumulation then removed trees ($F = 3.90$, $P < 0.027$; Fig. 3C). Similarly, resistant trees had 157% higher soil N accumulation (g m^{-2} yr^{-1}) than susceptible trees and 140% higher soil N accumulation than removed trees ($F = 5.25$, $P < 0.009$; Fig. 3D). At the tree-level, when age was taken into account, both C and N accumulation rates were significantly higher beneath resistant and removed trees than they were beneath susceptible trees (Fig. 3E, F). Annual soil C accumulation was 111%, or about 9 g yr^{-1} higher beneath resistant relative to susceptible trees, but there was no difference between resistant and removed trees ($F = 7.11$, $P < 0.0002$; Fig. 3E). Annual soil N accumulation was about twice as high (0.5 g yr^{-1} higher) beneath resistant trees than susceptible trees, but resistant and removed trees were similar ($F = 4.823$, $P = 0.012$; Fig. 3F). The piñons sampled were still juvenile, thus they had not had an ontogenetic shift that might have indicated a large change in growth and C allocation. In addition, these trees grew very slowly (thus, were small, see Fig. 1A) and had low C inputs and were enmeshed in an ecosystem that was constrained by moisture (Classen et al. 2005). As a reference, soils collected at the same time from inter-tree areas (areas between trees that are void of vegetation), had 78% lower soil C pools (380 ± 69 g C m^{-2}) and 83% lower soil N pools (18 ± 2 g N m^{-2}) than soils collected beneath the resistant piñons (Classen et al. 2006, 2007*b*). Thus, tree

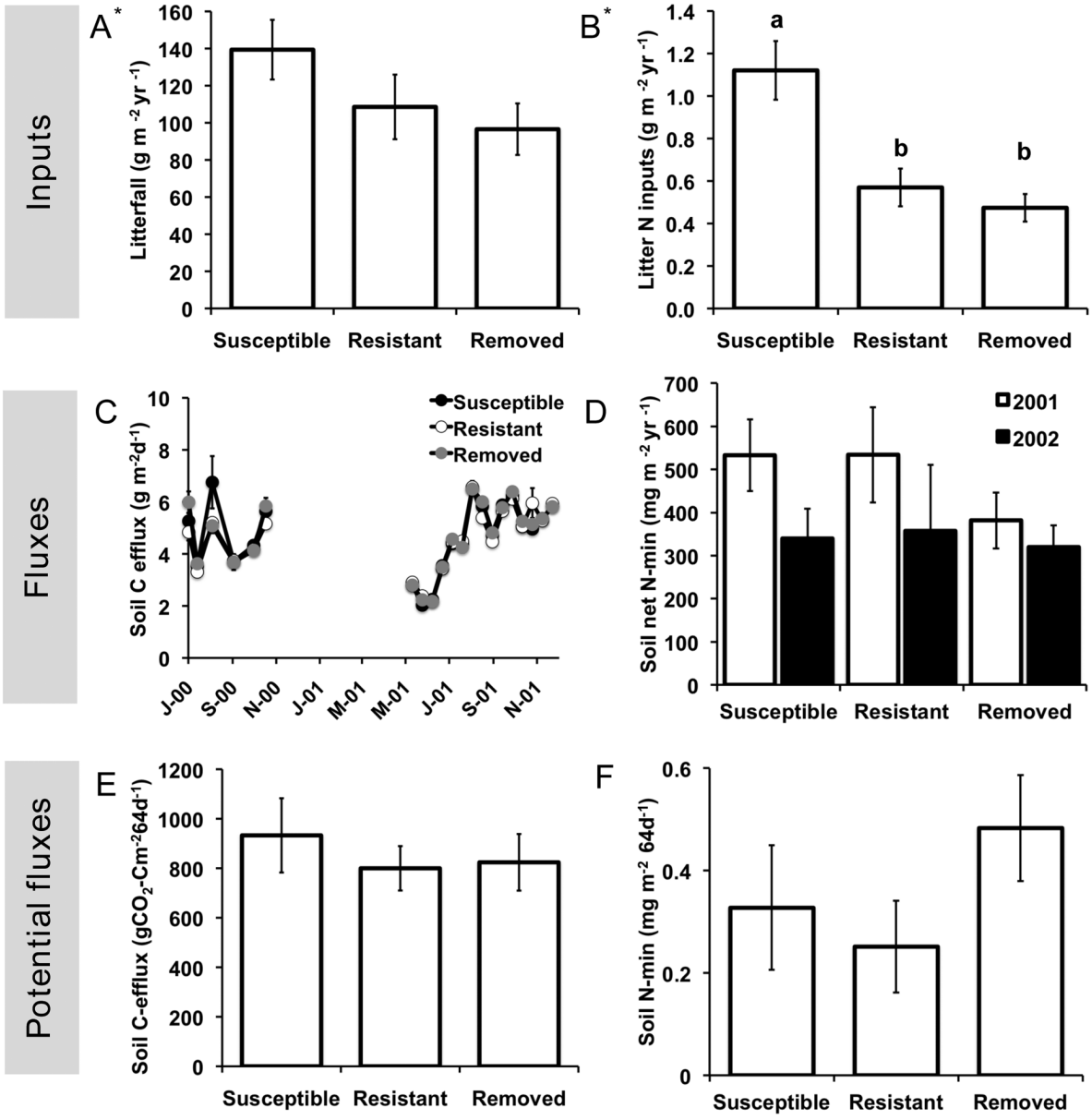

Fig. 2. Scale- susceptible, resistant, and removed tree impacts on: litterfall carbon (C) inputs over one year (A), litterfall nitrogen (N) inputs over one year (B), in situ soil C efflux over two growing seasons (C), in situ soil net N mineralization over two years (D), soil C efflux (e.g., microbial activity) over 64 days of incubation under laboratory conditions (E), and soil net N mineralization over 64 days of incubation under laboratory conditions (F). Herbivory increased litterfall N inputs (B) by over 50%, but did not significantly alter any other input or flux. Data are means ± SE (n = 20); different letters indicate significant differences among treatments ($P < 0.05$). Data in panels (A) and (B) are from Chapman et al. (2003).

establishment significantly increases soil C and N pools and is responsible for the majority of the accumulation of C and N in this ecosystem.

Interestingly, removal of herbivores from our trees significantly increased crown area even when the age of the tree was taken into account. When scales were removed, crowns of piñons were, on average, 54% larger per year than

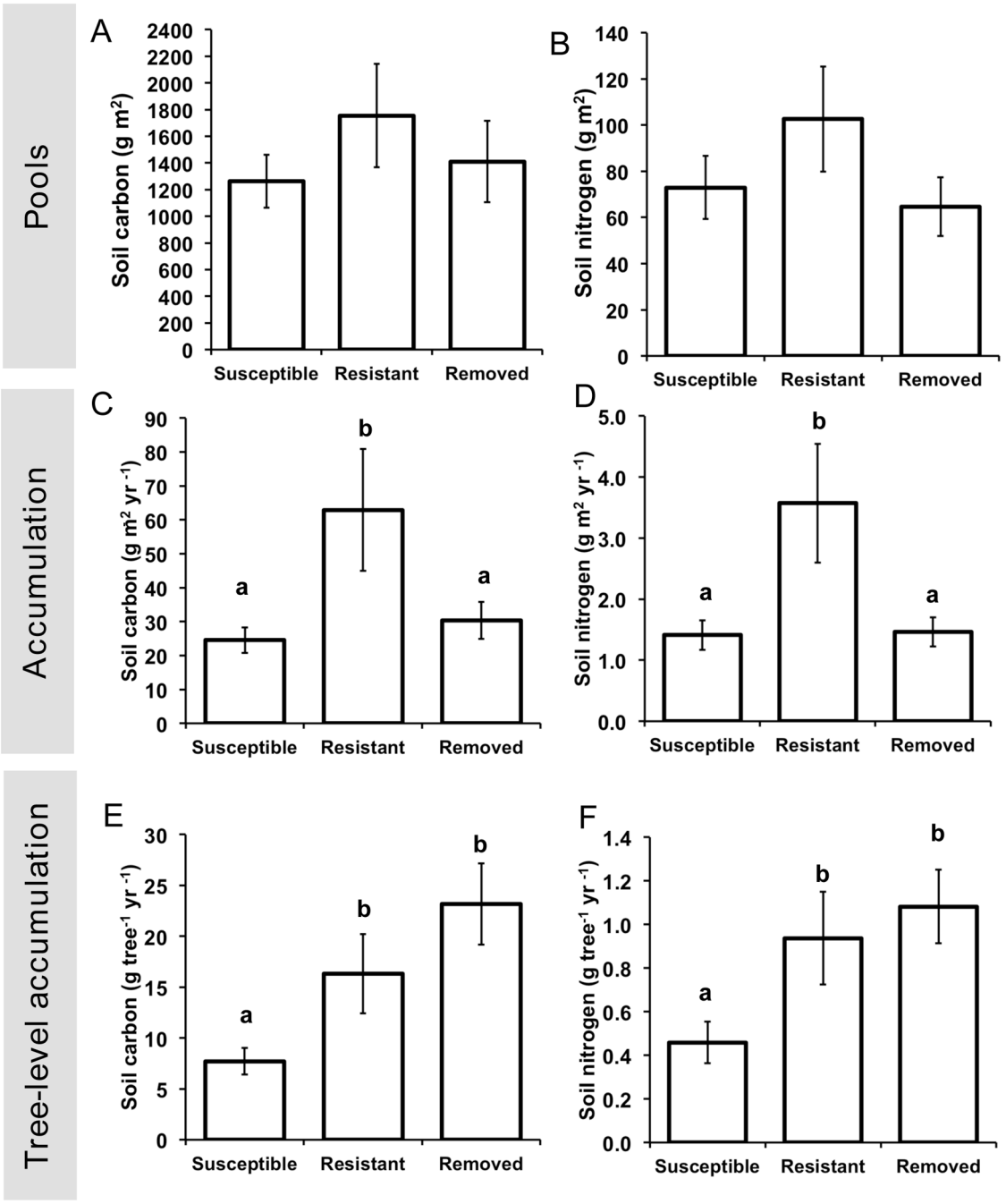

Fig. 3. Scale-susceptible, resistant, and removed tree impacts on: soil C pools (A), soil N pools (B), soil C accumulation (C), soil N accumulation (D), tree-level soil C accumulation (E) and tree-level soil N accumulation (F). Tree-level accumulation represents the amount of soil C or N that accumulated in the footprint of a given study tree over its lifetime from establishment to the present (i.e., approximately 50 years). While herbivory had no impact on pools (A and B), it significantly altered accumulation of soil C and N (C and D), especially when the impacts of herbivores on tree growth were taken into account (E and F). Data are means ± SE ($n = 20$); different letters indicate significant differences among treatments ($P < 0.05$).

susceptible or resistant crowns ($F = 9.3271$, $P = 0.0004$). On average, susceptible, resistant and removed trees had crown growth rates of 0.0085, 0.011, and 0.020 per year, respectively.

Discussion

Herbivore susceptibility traits as experimental tools for studying nutrient fluxes

Insect herbivores at this long-term study site had a striking impact on piñon canopy architecture, reducing leaf area index by 40% (Fig. 1; Classen et al. 2005). In addition, herbivores doubled N inputs as needle litter due to early needle abscission and associated incomplete N resorption (Chapman et al. 2003), decreased tree crown interception, and significantly increased soil temperature and moisture (Classen et al. 2005). Given these findings, we predicted herbivores would have dramatic impacts on C and N cycling pools and fluxes in this woodland ecosystem (Fagan et al. 2005, Knight and Chase 2005). Our long-term herbivore removal experiment allowed us the unique opportunity to examine how herbivore presence, absence, and removal alters C and N accumulation beneath trees that were likely the first colonizers at this site.

In spite of herbivore-driven changes in input quality and microclimate, over two years of study, we detected no differences in C or N soil fluxes beneath susceptible, resistant, and removed trees. The difference between fluxes and accumulation emerged because herbivory slowed tree growth rate, and in doing so, decreased C and N accumulation on the landscape. Thus, on the decadal time scale, scale impacts on C and N accumulation were amplified in soils beneath susceptible trees, thus slowing soil and ecosystem development.

Herbivore influences on soil N-fluxes

Contrary to our hypothesis that herbivore-increased litterfall N inputs would lead to increased soil N-mineralization, we saw no difference in net N-mineralization rates among herbivore treatments, across seasons, or between years (Fig. 2). Even when soil moisture and temperature were at favorable levels during a laboratory incubation, thus removing the influence of microclimate, we still found no differences in potential N mineralization among susceptible, resistant, and removed trees (Fig. 2). One possible explanation for a disconnect between increased litterfall N inputs and unchanging N mineralization is an altered microbial community beneath susceptible trees, which yields N mineralization rates similar to resistant trees. However, there were changes in potential extracellular enzyme activities, lower microbial biomass, and decreased mycorrhizal fungal colonization in soils under susceptible relative to resistant trees (DelVecchio et al. 1993, Gehring and Whitham 1995, Gehring et al. 1997, Classen et al. 2006). Mycorrhizal N-mineralization could be disproportionally important in this ecosystem due to its high aridity, which may decrease the presence of heterotrophic N-mineralizing bacteria and fungi (Langley et al. 2006); an area for further exploration.

Susceptible trees have decreased interception and thus increased canopy throughfall relative to resistant trees; therefore susceptible trees may have higher rates of leaching from the litter layer. Susceptible trees tend to have lower root biomass (Classen et al. 2007*a*) and fewer mycorrhizal mutualists (Gehring et al. 1997), which may lower N uptake. Additionally, the soils at our site are porous and have a very coarse texture, thus leaching losses could be greater in susceptible than resistant trees (Classen et al. 2007*b*), which could lead to equivalence in measured N mineralization rates, despite increased N inputs. However, we did not see evidence for increased leaching in the resins attached to the bottom of our mineralization cores. Thus, it is more likely that mineralization rates, across all tree types at our site, were constrained by water availability rather than input quality.

Herbivore influences on soil C-fluxes

We predicted that decreased standing biomass of scale-susceptible trees would lead to decreased needle litterfall and soil C-efflux rates. However, again, we found no difference in total needle litterfall, or laboratory or field C-efflux rates over two years. Scale insects cause all but the current year needles to abscise, thus opening up the individual tree crowns to light interception and decreasing shading for potential new shoots. Thus, needle litterfall rates could be higher (as a

proportion of total biomass) on susceptible trees because an increase in light interception may allow for the presence of more shoots that have a cohort of needles abscising each year (Chapman et al. 2003). This increase in shoot number may have generated statistically equivalent litterfall rates for resistant and susceptible trees (Fig. 2). Despite lower root, microbial, and mycorrhizal biomass (Gehring et al. 1997, Classen et al. 2006, Classen et al. 2007*b*), soil C efflux rates beneath susceptible trees may equal those beneath resistant trees due to increased soil moisture, the major limitation of C efflux in these semi-arid ecosystems (Conant et al. 1998, Classen et al. 2005). Regardless of the mechanistic explanations, over the two-year duration of our studies following 15 years of scale removal experiments from susceptible trees, we were unable to detect an impact of insect herbivory on soil C inputs and losses, despite dramatic reductions in piñon biomass.

Herbivore influences on soil C and N pools and accumulation

Soils beneath susceptible, resistant, and removed trees did not differ in C and N concentrations (Fig. 3A, B). Herbivore susceptible trees produce higher aboveground N inputs, have higher root N concentrations (Classen et al. 2007*a*) and tend to have higher litter inputs (and thus C inputs) than herbivore resistant trees. However, susceptible trees show a trend towards lower root inputs. Perhaps these contrasting root and needle inputs result in soil N and C concentrations similar to those of herbivore resistant and removed trees. It is possible that belowground inputs, both root and mycorrhizal, have a disproportionate impact on soil C and N pools in this ecosystem.

The long-term (36–54 year) impact of herbivores on soil C and N accumulation rates in this woodland, as measured by dividing the pool numbers by the age of the tree, were significant; herbivores decreased both soil C and N accumulation rates at the tree-level by over 50% (Fig. 3E, F). Counter to the soil C and N pool data, soils beneath both herbivore-removed and herbivore-resistant piñons had higher C and N accumulation rates than herbivore-susceptible trees (Fig. 3E, F). As mentioned above, herbivore-susceptible trees had lowered ability to access nutrients from their surroundings and fix C, thereby potentially leading to the decreased in soil C and N accumulation beneath them. When herbivores were removed, piñon canopies became two times larger than herbivore-resistant piñons, suggesting a significant C-cost to herbivore resistance. These larger trees yielded greater accumulation rates of soil C and N when we scale from the landscape scale to the whole-tree level (Fig. 3C, D). Thus, the difference in the removal tree accumulation rates on a g m^{-2} yr^{-1} level and on a g $tree^{-1}$ yr^{-1} is due to the higher growth of herbivore-removed trees. On the landscape scale, the presence of herbivores may be decreasing C and N accumulation beneath piñons by 50%, an especially significant finding in this young ecosystem.

Given the small soil C pool size in these arid woodland soils, small changes in C inputs and outputs due to herbivory (especially fluxes characterized by high daily and seasonal variation) may be undetectable over short time periods, but can build up over time to result in large differences in the soil development trajectory. Our capacity to measure these small short-term process changes in large soil pools, even with relatively robust replication ($n = 20$), may be insufficient, even in these young, C and N depauperate soils (Hungate et al. 1996). Furthermore, there was so much inter-annual and inter-seasonal variation in many of the process rates we measured that if both inputs and outputs of C and N are variable, the net change might be such that we cannot accurately assess it over our two-year study.

Because our study site is a primary successional ecosystem in a semi-arid woodland, we were able to assess: (1) changes in soil C and N accumulation and (2) an individual plant's "footprint" on soil development, and thus document the substantial impacts of insect herbivory on ecosystem soil development. The importance of piñon in regulating soil C and N pools in semi-arid landscapes is apparent from the larger C and N stocks beneath trees than in the canopy interspace (Neff et al. 2009, Reiley et al. 2010). The present study refines the 'islands of fertility' concept by documenting how insect herbivores can alter C and N pools and accumulation and possibly shape landscape dynamics over decades (Bishop 2002, Fagan et al. 2005, Knight and

Chase 2005). Because trees in this ecosystem often serve as nurse plants, the C and N stocks that develop beneath an individual will likely influence the future community of plants (Halvorson and Smith 2009). These findings suggest that other studies of herbivore impacts on ecosystem processes, which may not scale their results to include herbivore impacts on tree growth, might be similarly underestimating the significance of herbivores in influencing soil and ecosystem development.

Conclusion

In areas such as the southwestern US, climatic change is lengthening growing seasons, increasing temperature and drought events, and thus likely increasing plant and microbial stress (Breshears et al. 2005, Cregger et al. 2012). Together, these impacts can increase the influence insect herbivores have on ecosystem dynamics (Mueller et al. 2005, Sthultz et al. 2009). Already, the influences of outbreak herbivores, such as pine beetles, are reshaping western forests (Kurz et al. 2008, Pfeifer et al. 2011), so documenting long-term impacts of herbivory via field measurements and modeling is essential for predicting future productivity and resilience of ecosystems (Amiro et al. 2010). Herbivore impacts on soil C and N pools, coupled with large-scale die offs of piñon-juniper woodlands (Mueller et al. 2005, Huang et al. 2010), have the potential to significantly reduce the C and N stocks of these already depauperate ecosystems for decades. In sum, these data argue that chronic herbivory by insect herbivores can have large and sustained impacts on ecosystem development as well as future ecosystem trajectories.

Acknowledgments

We thank J. DeMarco, D. Guido, P. Cox, M. Lauretta, C. Andrekopoulos, R. Phylips, T. Ferrell, S. Hayden, M. Flynn, J. Abrums, M. Dickerson, K. Sides, B. Daughtry, B. Oerther, M. Classen, C. Classen, and D. Sanders for many hours of help in the field and the laboratory. K. Gehring and N. Cobb helped with establishment of the project and gave many helpful comments along the way. N.J. Sanders, J.A. Langley, and the ecosystem ecology group at UTK gave helpful comments on earlier versions of and discussion about this manuscript. This work was funded by the National Science Foundation (DEB 9816001 and DEB 0236204). A.T. Classen was funded, in part, by an American Association of University Women American Fellowship.

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