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The effect of nitrogen additions on bracken fern and its insect herbivores at sites with high and low atmospheric pollution

Michele Eatough Jones · Mark E. Fenn · Timothy D. Paine

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Abstract The impact of atmospheric pollution, including nitrogen deposition, on bracken fern herbivores has never been studied. Bracken fern is globally distributed and has a high potential to accumulate nitrogen in plant tissue. We examined the response of bracken fern and its herbivores to N fertilization at a high and low pollution site in forests downwind of Los Angeles, California. Foliage from the high pollution site had higher total N and nitrate than the low pollution site. Bracken fern biomass, foliar N and herbivore abundance were all affected by fertilization treatments. Biomass and herbivore responses were greatest during a year of high precipitation. N additions at the low pollution site were primarily associated with decreased fern biomass, and with transient impacts on herbivore abundance. N additions significantly affected bracken fern and its herbivores at the high pollution site where foliar N and nitrate decreased in response to N addition treatments, while biomass and herbivore abundance increased. High atmospheric deposition and fertilization were both associated with increased herbivore richness. Herbivore abundance was most impacted by fertilization during the early expansion of fern fronds. The most abundant chewing herbivore, a sawfly, was positively associated with plant nitrogen at the low pollution site, but negatively associated with plant nitrogen at the high pollution site, where concentrations of both total N and nitrate were high. While overall growth and herbivore impacts in this xeric location were limited, the variable response we observed associated with rainfall, may indicate impacts could be larger in more mesic environments.

Keywords Nitrogen deposition · *Pteridium aquilinum* · Bracken fern · Insect herbivore · San Bernardino Mountains

Introduction

Bracken fern (*Pteridium aquilinum* var. *pubescens* Underw.) is widely distributed in temperate zones throughout the world. Because of its invasive nature, it may become a serious weed problem in response to environmental changes due to pollution and climate change (Gordon et al. 1999; Werkman and Callaghan 2002; Whitehead et al. 1997). The objective of our research was to examine the response of bracken fern and its insect herbivores to atmospheric pollution in southern California. Atmospheric pollutants, particularly nitrogenous compounds and ozone are transported inland from urban areas of the Los Angeles basin, along the western slopes of the San Bernardino Mountains by prevailing weather patterns (Miller 1992).

In forests of eastern North America and Europe, N saturation has been associated with forest decline (Aber et al. 1995). In these mesic ecosystems, atmospheric deposition effects are believed to be primarily driven by base cation depletion, soil acidification and associated impacts (Fenn et al. 2006). In contrast, in California soils are not as highly leached because of the Mediterranean climate and base cation supplies are relatively high. Only

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M. Eatough Jones (✉) · T. D. Paine
Department of Entomology, University of California, Riverside,
CA 92521, USA
e-mail: michele.eatough@ucr.edu

M. E. Fenn
Forest Service, Pacific Southwest Research Station, Forest Fire
Laboratory, USDA, 4955 Canyon Crest Drive, Riverside,
CA 92507, USA

in the most polluted sites has severe soil acidification been observed. However, even in these instances base cation levels are still several fold higher than in polluted forests of Europe and eastern North America (Fenn et al. 2006). Because of the higher buffering capacity and base saturation of mixed conifer forests in California, nutrient effects of N deposition are of much greater concern than acidification effects (Fenn et al. 2010; Takemoto et al. 2001).

Bracken fern has a high potential to accumulate nitrogen (N) in plant tissue compared to other plants in the same habitat. Gerloff et al. (1966) found bracken fern had higher concentrations of N compared to other plants growing in northern dry forests. Stams and Schipholt (1990) found higher concentrations of nitrate in bracken foliage compared to other plants in forests impacted by atmospheric N pollution. In the San Bernardino and Sierra Nevada Mountains, total foliar N and nitrate were higher in bracken fern from polluted sites compared to less polluted sites (Fenn et al. 1996, 2003). Foliar nitrate of bracken fern was also much higher than for oak and pine in the San Bernardino Mountains. (Fenn et al. 1996). Nitrogen enrichment through atmospheric deposition has been shown to affect herbivore performance on a variety of plants (Throop and Lerdau 2004). When bracken fern herbivores from several sites along the pollution gradient in the San Bernardino Mountains were examined, herbivore communities at high pollution sites were characterized by a higher abundance of chewing insects (Eatough Jones and Paine 2006).

The objective of this study was to further examine the role of increased N deposition on bracken fern and its herbivore communities. We simulated increased N deposition through N additions at sites in the San Bernardino Mountains with different atmospheric pollution inputs. This is the first study to our knowledge to examine the effects of N amendments on herbivores of bracken fern. We expected that fertilization treatments would increase foliar nitrogen in bracken fern at both the high and low pollution sites. Increased foliar nitrogen was expected to affect bracken fern herbivores. If differences in the abundance of chewing herbivores previously observed at these sites were associated with atmospheric nitrogen inputs, fertilization treatments should increase the abundance of chewing herbivores, particularly at the low pollution site.

Methods

Sites and nitrogen addition treatments

Bracken fern plots were established in the fall of 1997 at two locations in the mixed conifer zone of the San Bernardino Mountains (San Bernardino County, CA) along a west/east gradient for N deposition and ozone. The western-most site at Camp Paivika (HIGH; 34°14′05″ N, 117°19′25″ W) has been impacted by high levels of N deposition while the more eastern site at Barton Flats (LOW; 34°10′10″ N, 116°54′25″ W) has received lower input from atmospheric N (Table 1). The dominant overstory species at the high pollution site are ponderosa pine (*Pinus ponderosa* Laws.), California black oak (*Quercus kellogii* Newb.) and incense cedar (*Calocedrus decurrens* (Torr.). The overstory at the low pollution site is dominated by ponderosa pine and the closely related Jeffery pine (*Pinus jeffreyi* Grev. & Balf.), California black oak and white fir (*Abies concolor* Gord. & Glend.). The parent material of soils in both areas is partially weathered or decomposed granite.

Three 10 m × 10 m bracken fern plots at each site were marked with a standard metal fence post at each corner and fertilized with Nitroform® (BFC Chemicals, Wilmington, Delaware) slow-release fertilizer (38-0-0) at a rate of 150 kg N ha^{-1} year^{-1}. This is a granular urea-formaldehyde fertilizer designed for dry application. Fertilizer was applied with a hand held spreader once each fall, beginning in 1997 and continued for the duration of the study. The slow release fertilizer was applied in autumn or early winter to simulate the pulse of N input as winter rains wash summer deposition off leaf surfaces into the soil. Corners of three unfertilized control plots of bracken fern were also marked with standard metal fence posts. Fern plots were chosen in relatively open areas with partial shade from nearby trees, but not directly under tree canopies.

Sample collection

Foliar insects and foliage were collected four times during each growing season (1998 through 2000). Sampling dates were determined by plant phenology. The first sample was collected as fern stems were elongating and fronds were uncurling (May). The second sample for fern was collected during mid expansion, after the main stem was elongated, but while fronds were still expanding (late May to early June; 1999 and 2000 only). A third sample was collected in midsummer at full leaf expansion (June to early July). The fourth sample was collected in summer at the first sign of yellowing as senescence began (August to early September).

The above ground growth of bracken fern was bagged and removed for all stems growing within four 0.5 m × 0.5 m areas randomly selected within each plot, for a total of 12 fern samples for each treatment. Collected foliage was stored on ice for transport and stored in an ultracold (−65°C) freezer in the laboratory. Insects were manually removed from the foliage, and identified to

Table 1 Site comparison for LOW and HIGH ambient air pollution

		LOW	HIGH
Elevation (meters)		1,900	1,600
Long-term average annual precipitation (mm)[a]		641	963
Yearly precipitation (mm)[a]	1998	1,055	1,259
	1999	317	392
	2000	582	704
Average annual temperature (°C)[b]		10.6	12.9
Ozone (ppb)[c]	Summertime 24-h hourly average	60.9	67.5
	Summertime average peak values	91.3	101.6
Throughfall nitrogen deposition[d]: kg N ha^{-1} $year^{-1}$		8	71

[a] Precipitation from PRISM Climate Group, Oregon State University, http://www.prismclimate.org. Average annual precipitation calculated from 1895 to 2007

[b] From (Fenn et al. 2005)

[c] Summer ozone concentrations were obtained from continuous ozone monitors at Crestline (California Air Resources Board, public information) and Barton Flats (Atmospheric Deposition Group, USDA PSW Research Station) for June through September 2002

[d] N deposition data were obtained from bulk ion exchange throughfall collectors (Fenn and Poth 2004). Data was collected for 2–5 years from 2000 to 2005 (Fenn et al. 2008)

family and morphotaxa. Morphotaxa were assigned based on visually distinct appearances. Herbivore abundance for each sample was recorded as individuals per 0.5 m^2 land area. This collection method emphasized the more stationary components of the herbivore community, and likely missed mostly highly mobile feeders, especially after fern fronds were fully expanded. However, comparison with sweep samples and visual inspections in the field indicated the most abundant herbivores were more sedentary feeders and present in the same relative proportions (Eatough Jones, unpublished data).

Herbivore morphotaxa were pooled for all sampling periods for 1998 through 2000 within each site and N addition treatment for richness estimates. This minimized the effects due to clumped species distributions and the small number of samples. Species richness was calculated using EcoSim (Gotelli and Entsminger 1997). Richness estimates were calculated based on rarefaction curves generated by EcoSim, with 1000 iterations of Monte Carlo-style simulations from the pooled herbivore communities. To standardize sampling intensity among treatments, herbivore richness was compared at the level that corresponded to 80% of the abundance for the treatment with the fewest herbivores for each sampling period. Significant differences between treatments were assessed from the 95% confidence intervals determined from Monte Carlo iterations.

Herbivore damage was estimated quantitatively. Individual leaves were selected randomly after insects had been extracted from the collected foliage. Enough leaves were chosen to fill 20 cm × 27 cm grid. Foliage was laid out and covered with a 4 mm × 4 mm squares printed on a clear acetate sheets. Ten random transects were selected, and each grid point over foliage was scored as damaged or undamaged. Damaged points were points where there was evidence of feeding from chewing herbivores and miners.

Plant characteristics

Foliar analyses were conducted using plant tissue collected during insect sampling. Immediately after insects were removed from foliage, all leaves from each sample were weighed to determine fresh sample weight. Plant samples were dried at 50°C for 3 days and weighed again to calculate dry weight. Oven-dried plant tissue was ground with a Wiley mill to pass through a 60 mesh screen. Sample analyses performed by the Division of Agricultural and Natural Resources Analytical Laboratory at the University of California, Davis for total N was by Carlo Erba combustion (Pella 1990). Analysis for nitrate was by aqueous solvent extraction with 2% acetic acid and quantitative determination by flow injection analysis (Carlson et al. 1990; Wendt 1999). Total N was determined for samples collected during early expansion, mid expansion and during the period when leaves reached full expansion. Nitrate was determined for samples collected during the period when leaves reached full expansion but not for earlier sampling periods due to limited plant material.

Differences in herbivore abundance and plant characteristics among treatments and years within each site were tested by ANOVA with N addition treatment and year as main effects using SAS (2003). Contrasts within each site and sampling period had 12 samples collected at each of 2 levels of nitrogen fertilization for 3 years for a total of 72 observations. Samples were not collected during 1998 at mid expansion, so there were a total of 48 observations in this period. Because of limited plant material, analysis for total N and nitrate was not available for all data points. Due to missing values, the number of observations for total N at the low pollution site were n = 34 (early expansion) and n = 37 (mid expansion) and at the high pollution site n = 54 (early expansion) and n = 36 (mid expansion). Missing values for nitrate at full expansion gave n = 70 at the low pollution site and n = 66 at the high pollution site.

We conducted 2 analyses to evaluate herbivore response over the 3 year duration of the study. We determined the relationship of plant characteristics to abundant herbivore groups at each site using Pearson correlation coefficients. Significant correlations were evaluated at $\alpha = 0.05$. Herbivores chosen for individual comparisons were present at all sites and treatments, and were the most abundant herbivore taxa (over 500 individuals collected). For the two most common chewing herbivores, we also evaluated overall abundance and damage caused for data pooled across all 3 years.

Results

Plant characteristics

Fern biomass was significantly affected by N additions at the LOW site, and by N additions and year at the HIGH site (Table 2). Overall there was a trend for decreased biomass for fertilization treatments at LOW and a trend for increased biomass for fertilization treatments at HIGH (Fig. 1). In 1998, fern biomass (grams dry weight 0.5 m^{-2} land area) at the time of full expansion for unfertilized fern at LOW was significantly higher than for fertilized fern, and higher than biomass collected in other years. Fern biomass was highest in 1998 at the high pollution site, and higher for fertilized fern.

There were never any significant differences in total plant N for fern at the LOW site (Fig. 2). Plant nitrate was higher for LOW fern in 1998 compared to 1999 and 2000, but there were no differences among nitrogen addition treatments (Table 2). Plant N was significantly affected by N additions at all stages of expansion at the HIGH site (Fig. 2). In general, both plant N and nitrate were decreased by fertilization. Year of sample collection also influenced plant N at HIGH, particularly at full expansion (Table 2). Total N for control and fertilized fern was significantly different during mid expansion in 2000 and at full expansion in 1998 and 1999. Plant nitrate at full expansion was also higher for control fern compared to fertilized fern at HIGH, and significantly higher in 2000. Plant N was higher for HIGH plants compared to LOW plants for all years at mid expansion and at full expansion (Fig. 2).

Herbivore richness and abundance

A total of 54 herbivore morphotaxa and 10 024 individuals were collected on bracken fern. 11 taxa were collected in all treatment plots. HIGH had 26 unique morphotaxa, while LOW had 12 unique morphotaxa. The remaining 5 taxa were collected at both sites, but were not present in all treatment plots. For both HIGH and LOW, > 98% of all individuals collected were from morphotaxa common to both sites. Those herbivores present in high abundance for all treatments included thrips, mites, nectar-feeding ants, aphids, a rachis-mining Lepidoptera (Gelechiidae) and a foliage feeding sawfly (Tenthridinidae).

Throughout the growing season, fern herbivore communities were characterized by 2 or 3 dominant herbivores. Fern samples collected during early expansion were dominated by a rachis miner (50% of individuals collected) and nectar feeding ants (33%). The rachis miner (38%) and

Table 2 ANOVA statistics

	Stage	Low pollution site			High pollution site		
		F	*P*	Effects	F	*P*	Effects
Biomass	Full	5.7	<0.01	N	9.9	<0.01	N, year, N*year
Total N	Early	2.7	0.05		2.7	0.03	N
	Mid	2.1	0.12		7.1	<0.01	N, N*year
	Full	1.1	0.37		10.5	<0.01	N, year
Nitrate	Full	3.6	0.01	Year	3.6	0.01	N, year
Abundance	Early	7.4	<0.01	Year	6.3	<0.01	N, year
	Mid	3.9	0.01	Year	6.9	<0.01	Year, N*year
	Full	2.8	0.02	Year	8.5	<0.01	N, year, N*year
	Summer	1.9	0.10	Year	2.2	0.06	

Contrasts tested effects of level of N addition (N), year of sampling (year) and the interaction of N additions and year (N*year). Overall F is shown and significant contrasts listed

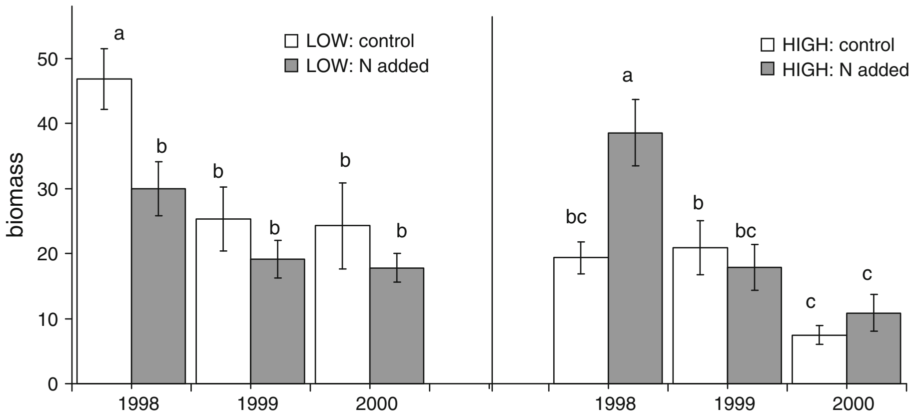

Fig. 1 Foliage biomass (mean and SE). Biomass (g dry weight foliage 0.5 m^{-2} land area) of bracken fern was measured when fronds reached full expansion for control and nitrogen addition treatments at sites with differing air pollution (LOW on the *left* and HIGH on the *right*). Within each site, *bars* with *different letters* were significantly different at $\alpha = 0.05$

sawflies (16%) were common at mid expansion. Free feeding sawflies (10%) were also common herbivores at full expansion. Aphids were also abundant by mid-expansion (38%) and remained abundant (74%) through the rest of the growing season.

There were no differences in herbivore richness among sites and treatments during early expansion or mid expansion (Fig. 3). For samples collected at full expansion, herbivore richness was significantly higher for the fertilized plots at HIGH compared to all other treatments. For samples collected during the summer, herbivore richness for control plots at LOW was significantly lower than richness for both HIGH treatments, and richness for LOW fertilized plots was significantly lower than for HIGH fertilized plots (Fig. 3).

There was rarely any difference in herbivore abundance at the low pollution site in response to nitrogen addition treatments (Fig. 4). Herbivore abundance did vary among years at LOW (Table 2). During early expansion, herbivore abundance was highest in 1998. Later in the growing season, herbivore abundance was highest in 1999. Bracken fern herbivores showed more differences related to fertilization treatments at the high pollution site, particularly during early expansion (Table 2). Total herbivore abundance at HIGH was higher on fertilized plots than control plots during early expansion (Fig. 4). Later in the season there were few differences among years or treatments at HIGH. The one exception was high abundance for control plots during 1999.

We compared damage to fern and abundance of individual herbivores for two of the most common herbivores, the rachis mining Lepidoptera and the free-feeding sawfly, pooled across all 3 years of sample collection (Table 3). Data was pooled for the period of peak abundance for each herbivore. The rachis miner was most abundant during early expansion while the sawfly was most abundant as fern fronds reached full expansion. While these herbivores were both present at mid-expansion, nearly all damage to fern fronds was made by only one taxon at early and full expansion. Abundance of both the rachis miner and the sawfly and damage to fern were higher at the high pollution site compared to the low pollution site. There were no significant differences in damage or abundance of rachis miners and sawflies between control and fertilization treatments within either site.

Relationship between plant characteristics and herbivore abundance

Abundance of the rachis mining Lepidoptera was never correlated with plant N at either site (Table 4). Sawfly abundance was significantly positively correlated with plant N at LOW during full expansion, but negatively correlated with plant N during mid expansion at HIGH. Aphid abundance was significantly negatively correlated with plant N at LOW during mid expansion, but never correlated with plant N at HIGH. Abundance of nectar-feeding ants was positively correlated with plant N at LOW during mid expansion, but negatively correlated with plant N during early expansion at HIGH.

Discussion

We chose to study the response of bracken fern herbivore communities to simulated atmospheric nitrogen deposition through N fertilization because bracken fern has shown a strong capacity to store excess N in foliage (Fenn et al. 1996; Stams and Schipholt 1990). Fern biomass per land area and fern herbivore abundance were affected by fertilization at both low and high pollution sites. In contrast, a similar fertilization study at these sites found no response to fertilization for herbivores on California black oak at the low pollution site (Eatough Jones et al. 2008). Bracken fern herbivores may be more responsive to small changes in N allocation within plants or changing patterns of plant

Fig. 2 Foliage N and nitrate (mean and SE). Total N was measured at early, mid and full expansion. Soluble nitrate (ppm) was measured at full expansion. Each site (LOW (*left*) and HIGH (*right*) ambient air pollution) had control and nitrogen addition treatments. Within each site, *bars* with *different letters* were significantly different at $\alpha = 0.05$

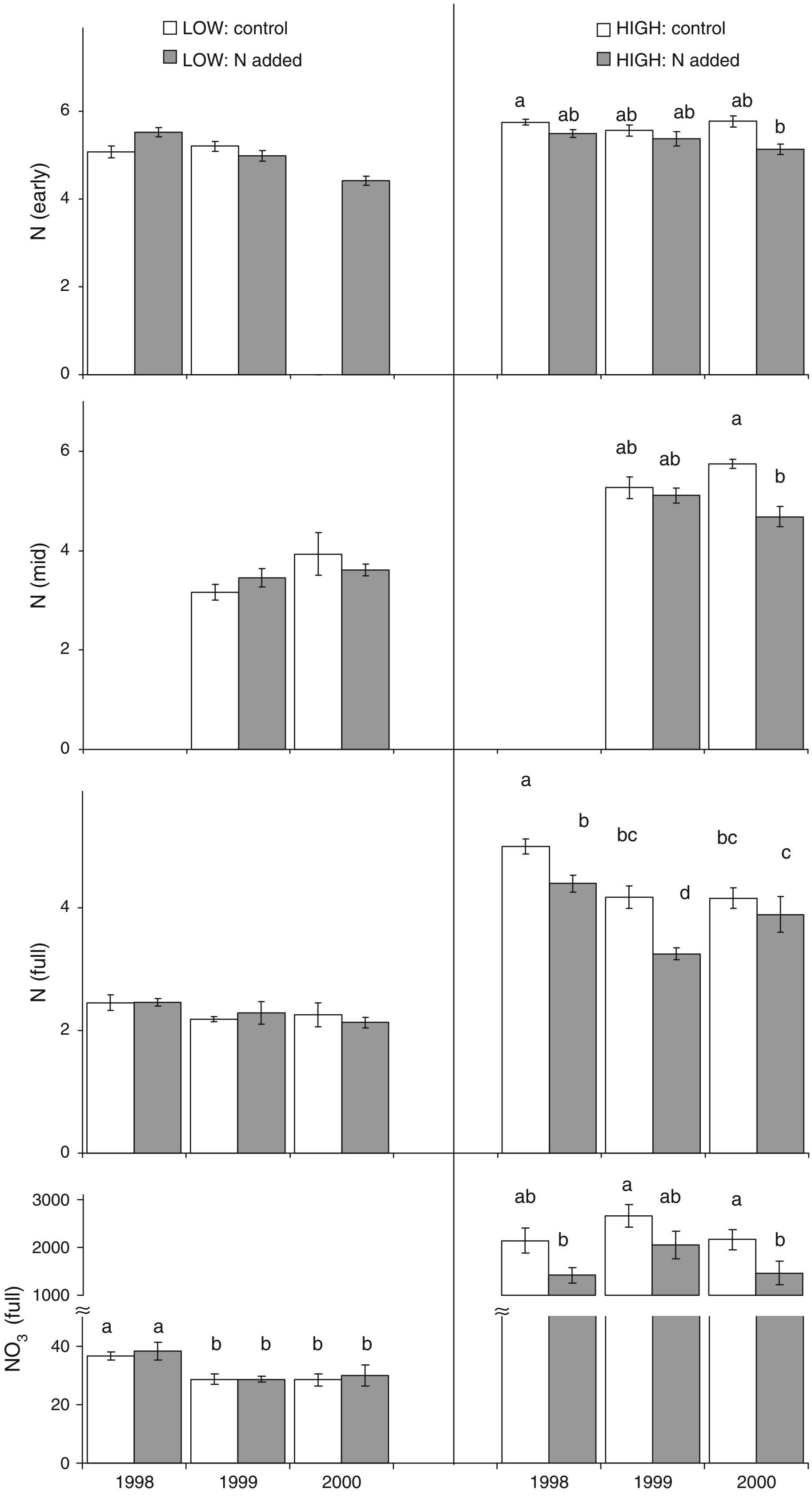

Fig. 3 Bracken fern herbivore richness and 95% confidence intervals. Herbivores were pooled within each sampling period for all samples collected 1998 through 2000. Each site (LOW and HIGH ambient air pollution) had control and nitrogen addition treatments. Within each sampling period, bars with *different letters* are significantly different as determined by 95% confidence intervals

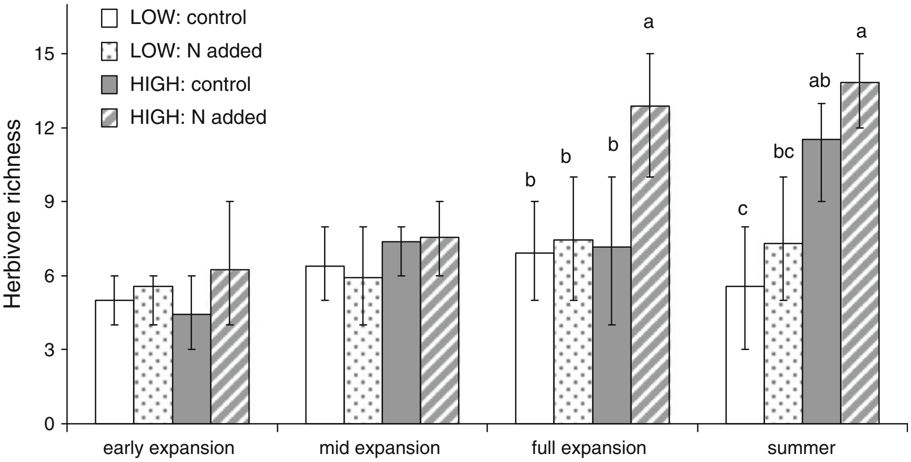

Fig. 4 Fern herbivore abundance (mean and SE). Total herbivore abundance for each sampling date and each year during early, mid and full expansion. Each site (LOW (*left*) and HIGH (*right*) ambient air pollution) had control and nitrogen addition treatments. Within each site, *bars* with *different letters* were significantly different at α = 0.05

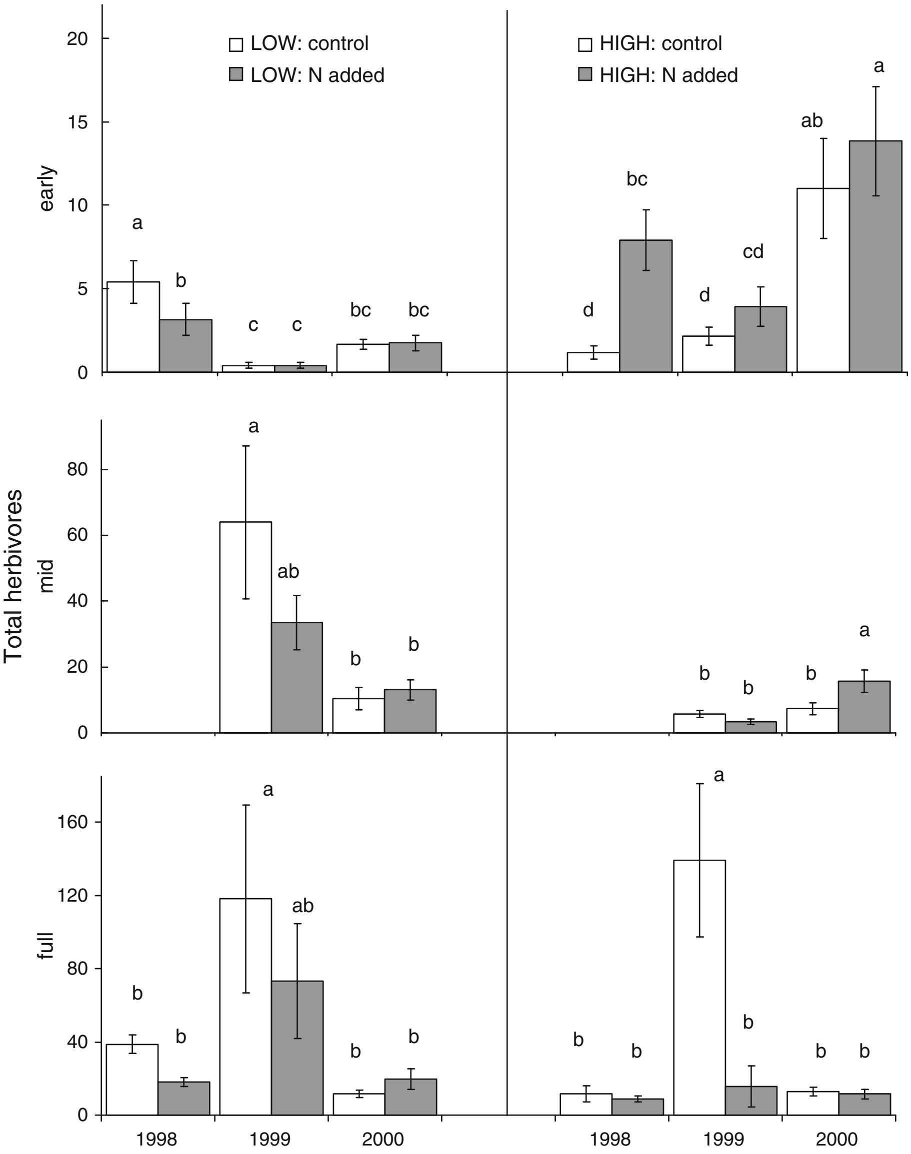

Table 3 Herbivore abundance (individuals per 0.5 m^2 land area) and associated damage (%) to fern fronds for two common herbivores at a low and high pollution site

Site	Treatment	Rachis miners	Damage (early)	Sawflies	Damage (full)
Low	Control	0.8 ± 0.2^{b}	3.0 ± 0.9^{ab}	1.7 ± 0.5^{b}	8.4 ± 1.0^{ab}
	N added	0.3 ± 0.1^{b}	0.7 ± 0.3^{b}	0.7 ± 0.2^{b}	7.3 ± 0.8^{b}
High	Control	3.7 ± 1.1^{a}	6.2 ± 1.7^{a}	5.6 ± 1.0^{a}	13.9 ± 2.6^{ab}
	N added	3.9 ± 0.7^{a}	5.6 ± 1.2^{a}	5.1 ± 1.1^{a}	15.3 ± 2.5^{a}
F		7.83	4.80	9.76	4.35
P		<0.001	0.003	<0.001	0.006

Data were pooled across years within each treatment. Rachis miners were present during early expansion. Sawflies were present as bracken fern reached full expansion. Contrast compared site (Low and High ambient air pollution) and level of N addition. Significant differences are indicated within each column

Table 4 Pearson correlation coefficients for plant N with herbivore abundance

	Low pollution			High pollution		
	Early	Mid	Full	Early	Mid	Full
Rachis miner	−0.10	0.16	−0.07	−0.04	0.05	−0.03
Sawfly	−0.24	0.09	0.37*	−0.07	−0.43*	0.10
Aphid	−0.04	−0.46*	−0.05	−0.01	0.12	−0.01
Ant	−0.02	0.47*	0.15	−0.27*	−0.23	0.02

LOW: n = 34 early, n = 37 mid, n = 72 full. HIGH: n = 54 early, n = 36 mid, n = 72 full. Correlations for herbivores were performed across all years and within each site. $^{*}P < 0.05$

growth at the low pollution site. Both fern and oak herbivores showed responses to fertilization at the high pollution site, indicating that further N pollution inputs will continue to alter plant-insect interactions in southern California forests.

Plant nutrient response

Contrary to our expectations, we found that both total N and plant nitrate were decreased in foliage of fertilized plants at the high pollution site. In 1998, when plant biomass was very high for fertilized fern, it is possible that the common phenomenon of growth dilution may have contributed to lower plant N for fertilized fern at the high pollution site. However, biomass responses at the high pollution site in 1999 and 2000 don't support this as a mechanism to explain overall decreases in plant N and nitrate. Another possible factor may be that fertilization at the high pollution site increased microbial immobilization of N, and consequently the N available for assimilation by bracken fern may have decreased. Although data collected in this study cannot conclusively demonstrate microbial mobilization, evidence from other studies conducted in the San Bernardino Mountains suggests the possibility (Fenn et al. 2005).

There is much more litter in the forest floor and higher organic matter content in the A soil horizon at the high pollution site compared to the low pollution site (Fenn et al. 2005). Litter and soil organic matter at the high pollution site may provide abundant C for microbial immobilization of N. That, in combination with the added N, may stimulate microbial growth resulting in less N available for plant uptake in fertilized plots at the high pollution site. It is common for a large fraction of added N to be incorporated into the organic horizon (Fenn et al. 1998), and N immobilization can be much greater in the organic horizon compared to mineral soil (Johnson et al. 2000). Aber et al. (1998) reported from a literature review that two-thirds of N added in N amendment studies ended up in the soil organic layer without passing through aboveground plant tissues.

Although both oak and pine trees at the high pollution site showed increased radial growth in response to fertilization treatments (Fenn and Poth 2001), fern may take a much greater fraction of N from the A horizon and humus horizons than do the forest trees. As a result, microbial immobilization may affect N availability for fern more than for pine and oak, which are deeply rooted in the mineral soil and weathered bedrock (Witty et al. 2003). Neither net N mineralization nor net nitrification in the mineral soil was affected by N additions at the high pollution site (Fenn et al. 2005). However, fertilization resulted in lower nitrate levels in forest soils at the high pollution site (Fenn et al. 2005). Additionally, high rates of gross NH_4 immobilization in the organic layer at the high pollution site (ME Fenn, unpublished data), and higher total C in fertilized soils, also suggest that increased microbial immobilization of N may be taking place in the upper mineral soil and organic horizon of fertilized plots at the high pollution site. It is likely that a combination of differences in available C in the mineral soil, litter layer depth, microbial immobilization, understory plant diversity and precipitation account for the different responses of fern to fertilization at these two sites.

Plant growth response

Nitrogen fertilization studies with bracken fern have often shown little positive effect on plant growth (Gordon et al. 1999; Werkman and Callaghan 2002; Werkman et al. 1996; Whitehead et al. 1997). Our study suggests that high N availability may have even decreased fern biomass. Although biomass was higher for fertilized fern at the high pollution site, N in plant tissues and soil NO_3 (Fenn et al. 2005) were actually decreased by N additions, so that plants from control plots at the high pollution site with higher foliar N had lower biomass production. Fern biomass from fertilized plots at the low pollution site was consistently lower than for control plots. This was particularly evident during a year of high precipitation. There was an El Niño event during the winter of 1997–1998. Rainfall for 1998 in the San Bernardino Mountains was much higher than rainfall for 1999 and 2000 (Table 1). Gordon et al. (1999) found that drought had a greater impact than N supply on fern growth, and decreased both frond density and height. Our results also suggest that water availability is more important than N availability for fern biomass production.

We would expect that N fertilization and added precipitation from the El Niño would have increased fern growth (e.g. Waring and Cobb 1992), particularly at the low pollution site. We can only speculate that the additional precipitation stimulates microbial N immobilization. It is possible that immobilization may be promoted by the slow release fertilizer we used which depends on microbial release of the N, so that at the N-poor site microbes outcompeted the plants for N. A negative growth response to N fertilization was also found for trees at the low pollution site (Fenn and Poth 2001).

Other studies of the effects of N fertilization on bracken fern have used rates equivalent to 50 kg N ha^{-1} $year^{-1}$ (Gordon et al. 1999; Werkman and Callaghan 2002; Werkman et al. 1996; Whitehead et al. 1997) compared to the rate of 150 kg N ha^{-1} $year^{-1}$ that we used. Our high rate of fertilization may be why we observed decreased biomass while other studies have not. However, deposition in some areas of southern California already exceed 50 kg N ha^{-1} $year^{-1}$. The current rate of atmospheric deposition at our high pollution site is 71 kg N ha^{-1} $year^{-1}$, and average annual fluxes under pine canopies as summer deposition is washed off by winter rains are ca. 130 kg N ha^{-1} $year^{-1}$ (Fenn and Poth 2004). However, with variable rainfall among years, different soil N characteristics between our sites, and possible interference from choosing a fertilizer that required microbial release, we cannot confidently presume that the observed decrease in fern biomass was due directly to N fertilization. The interactions between rainfall and atmospheric nitrogen deposition warrant further investigation.

Herbivore responses

We found evidence that plant N status does influence abundance for bracken fern insect herbivores. Nitrogen deposition has previously been shown to affect herbivore performance (Throop and Lerdau 2004). Most insects respond positively to increased plant nitrogen (Kyto et al. 1996; Mattson 1980; Waring and Cobb 1992). Similarly, we found that sawfly abundance was positively correlated with plant N at the low pollution site, and chewing damage to fern as well as abundance of sawflies and a rachis mining Lepidoptera were both higher at the high pollution site compared to the low pollution site. Bracken fern has many carbon-based defensive compounds including tannins (Jones 1983). This may affect sawflies more than rachis miners since tannin concentrations are low in expanding fronds while miners are active and high in mature foliage when sawflies are present (Jones 1983; Tempel 1981). Fertilization may decrease concentrations of tannins, and increase palatability for insect herbivores (Feeny 1970).

However, abundance of sawflies was negatively correlated with plant N at the high pollution site. Differences in the association of plant N and herbivore abundance for these two sites may be related to the large difference in plant N status between sites and the high nitrate concentrations in fern fronds at the high pollution site. Total plant N and plant nitrate were positively correlated. Foliar nitrate at the high pollution site was nearly 2 orders of magnitude higher than nitrate at the low pollution site. High plant nitrate can be a feeding deterrent for many insect herbivores (Jansson and Smilowitz 1985; Manglitz et al. 1976). This may be particularly important for chewing herbivores such as sawflies. High nitrogen availability may also be associated with an increase in nitrogen based plant defenses (Bryant et al. 1983). Bracken fern has a broad array of secondary compounds associated with anti-herbivore properties (Jones 1983) including the enzyme thiaminase which may increase with high nitrogen availability. Conversely at the low pollution site, where plant nitrate is much lower than at the high pollution site, higher plant N may make plants a more favorable resource for sawflies.

Nectar feeding ants and aphids may be responding to differences in plant growth, timing of nectar production or nectar quality. Fertilization is known to affect amino acid composition for some plants (Gardener and Gillman 2001) and amino acids in nectar can affect ant attraction (González-Teuber and Heil 2009). However, bracken fern has not been shown to benefit from ant attraction to extrafloral nectarines (Rashbrook et al. 1992) and the effects of fertilization on nectar of bracken fern are unknown.

Aphids are likely avoiding plant nitrate, which is stored in the vacuole, by feeding directly from phloem. Aphids, which have peak abundance later in the season, may be preferentially colonizing larger, more expanded plants at the low pollution site. Further examination of changes in amino acids and secondary compounds of bracken fern may provide further insight for the relationship between changes in plant N status and insect herbivore responses.

Conclusions

In spite of bracken fern's global distribution, common noxious weed status and potential to respond to environmental change, the impact of atmospheric pollution on bracken fern herbivores has never been studied. We found indications that atmospheric N inputs have impacted bracken fern herbivores in the San Bernardino Mountains of California.

The response of sawflies and a rachis mining Lepidoptera to N additions may warrant further investigation. Rachis miners can inhibit fronds from fully unfurling and affect leaf surface area in mature fern (Eatough Jones, personal observation). Sawflies were the only free-feeding herbivore we observed to have a major impact on removing leaf area through herbivory. We did not observe damage from herbivory by mammals during this study. These two insect herbivores have the greatest likelihood of causing damage to fern in the San Bernardino Mountains, potentially reducing frond surface area and impacting ground cover in fern stands.

While we did see effects of fertilization within each site, there were much stronger differences in herbivore abundance between sites. Additionally, environmental differences from year to year had a stronger influence. We did see indications that bracken fern response to nitrogen inputs may vary depending on rainfall, with the strongest responses being observed in a year of high rainfall. Bracken fern has a broad geographical distribution and is a noxious weed in many areas with more mesic climates compared to southern California (Taylor 1990). Our study suggests that the impacts of atmospheric nitrogen deposition on bracken fern plant N may be more pronounced in more mesic climates. The interactions between rainfall and atmospheric nitrogen deposition warrant further investigation.

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