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The major features of an infestation by the invasive weed legume gorse (*Ulex europaeus*) on volcanic soils in Hawaii

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Abstract Gorse (*Ulex europaeus*) infestation occupies over 4,000 ha of agriculture and conservation lands on the southeastern slope of Mauna Kea on the Island of Hawaii. The aim of this investigation is to identify ecological features associated with this weed invasion by comparing the gorse-infested areas to the surrounding uninfested areas of this landscape. The soils within the gorse infestation are more acidic, resulting in higher levels of KCl-extractable Al and lower levels of Mehlich III-extractable Ca, Mg, Mn, and Zn. Yet, gorse accumulates higher concentrations of Ca, Zn and, Cu than the kikuyu grass (*Pennesitum clandestinum*), which is ubiquitous throughout the site. The Ca:Al and Mg:Al molar charge ratios of the soils are lowest within the epicenter of the gorse infestation, while the molar ratios are highest in the gorse apical stem tissues. All gorse plants are nodulated and have higher nitrogen contents than the surrounding kikuyu grass. Furthermore, the $\delta^{15}N$ of the gorse stem tissues approaches 0‰, suggesting that nitrogen is being symbiotically fixed from the atmosphere. Characterization of the *Bradyrhizobium* isolated from gorse nodules shows similarities and distinctions to *Bradyrhizobium* isolated from the endemic legume koa (*Acacia koa*) within the same location. Population densities of the indigenous *Bradyrhizobium* are higher within the gorse rhizosphere than the kikuyu grass. Soil acidification, nutrient depletion, and symbiotic nitrogen fixation distinguish gorse-infested areas from the surrounding uninfested areas. These observations suggest that gorse has a competitive advantage over kikuyu grass under conditions of soil nutrient deficiency.

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Introduction

Gorse (*Ulex europaeus*) is a woody legume endemic to the heathlands of western Europe and coastal areas of the Mediterranean (Allen and Allen 1981). It is also a weed species that threatens natural habitats and agroecosystems in many other parts of the world, especially in Australia, Brazil, Canada, Chile, England, Germany, New Zealand, India, Spain, and the United States (Gaynor and MacCarter 1981; Holm et al. 1977; Hoshovsky 1986). Gorse is officially classified as a noxious weed species in the State of Hawaii. First observed on the Island of Maui in 1910, gorse infestations have reached epidemic proportions on high-elevation agriculture and conservation lands, on both the islands of Maui and Hawaii (Motooka et al. 2003).

Gorse is an opportunist legume species that is adapted to disturbed landscapes with low fertility including logged areas and overgrazed pastures (Hoshovsky 1986; Matthews 1982; Parker 1984; Zabkiewicz 1976). In Hawaii, pasturelands have been the most vulnerable to gorse invasion. In 1992, an estimated 2,000 ha of pastureland in Humuula, which is located on the slope of Mauna Kea on the island of Hawaii, was infested with gorse. Today, this gorse infestation occupies over 4,000 ha (Mike Robinson 2004, personal communication). This infestation is directly adjacent to the Hakalau Forest National Wildlife Refuge and threatens to compete with the endangered flora and fauna in this protected habitat.

The invasive nature of gorse has been attributed to several physiological factors including mature cohort crowding that creates a dense thicket, long-term perennial growth, and a large persistent soil seed bank (Chater 1931; Ivens 1983; Hill et al. 2001; Lee et al. 1986). These qualities appear to play an important role in the successful establishment of gorse in Humuula as well. This gorse-infested location is a landscape of low floristic diversity. Besides gorse, the only remaining vegetation consists mainly of

kikuyu grass (*Pennesitum clandestinum*), an important pasture species in Hawaii. However, the dense gorse thickets appear to be more productive and also well adapted to this location. The land-use history of the Humuula landscape for the past 100 years includes logging of the highly valued koa tree (*Acacia koa*), conversion to pasture, and intense cattle grazing. As a result of these activities, this landscape became susceptible to gorse invasion and subsequent infestation. The objective of this investigation is to identify distinguishing ecological features associated with gorse infestation on the volcanic ash-derived soils in Humuula.

Materials and methods

Site description

The Hawaiian archipelago is one of the most isolated island groups in the world, being located in the middle of the Pacific Ocean and 4,000 km from the nearest continent (Juvik et al. 1998). The Island of Hawaii is the largest and the most southeastern island of this chain. The gorse-infested site in Humuula is located 1,900 m above sea level (asl) along the southeastern slope of Mauna Kea on the Island of Hawaii, and is adjacent to the Hakalau Forest National Wildlife Refuge (Fig. 1a). Current activities on this land include pasture grazing, salvage logging, and koa reforestation. This site shares many of the same environmental parameters described by Scowcroft et al. (2004). Mean daily air temperatures range from 9 to 14°C and mean annual rainfall is more than 1,500 mm year^{-1} (Giambelluca et al. 1986). The soils are derived from the volcanic ash of Mauna Kea. and are classified as a medial, amorphic, isomesic, dystric Haplustand, of the Laumaia series (C. Smith, personal communication). Kikuyu grass is the other dominant vegetation, with only minor establishments of other pasture grasses including *Anthozanthum ratum*, *Holcus lanatus*, and *Axonopus fissifoliusas* (Scowcroft et al. 2004). There are also a few isolated, mature koa stands within the area, but most are established within the ravines and waterways. There are two successional patterns of gorse infestation in Humuula: (1) heavy infestation of mature, impenetrable thickets, and (2) dispersed establishments of pioneer plants in the uninfested periphery. The mature gorse plants within the epicenter of the infestation are all over 2 m in height and are surrounded by mature cohorts within 1 m of each other. Gorse established within the uninfested periphery varied in size from 0.5 to 2 m in height and are established as individuals or as small clusters.

Experimental design

The gorse infestation in Humuula was accessed in December 2003. Four experimental units were established across the gorse infestation within the coordinate range of 155°20′22″–155°24′03″W and 19°44′00″–19°50′04″N (Fig. 1b). Each experimental unit contained two transects designating the gorse-infested epicenter (e) and uninfested periphery zones (p). Apical stem tissue, nodule, and soil samples were collected from eight mature gorse plants along each transect, with each sampled plant at least 50 m apart from each other. The epicenter transects, were at least 50 m

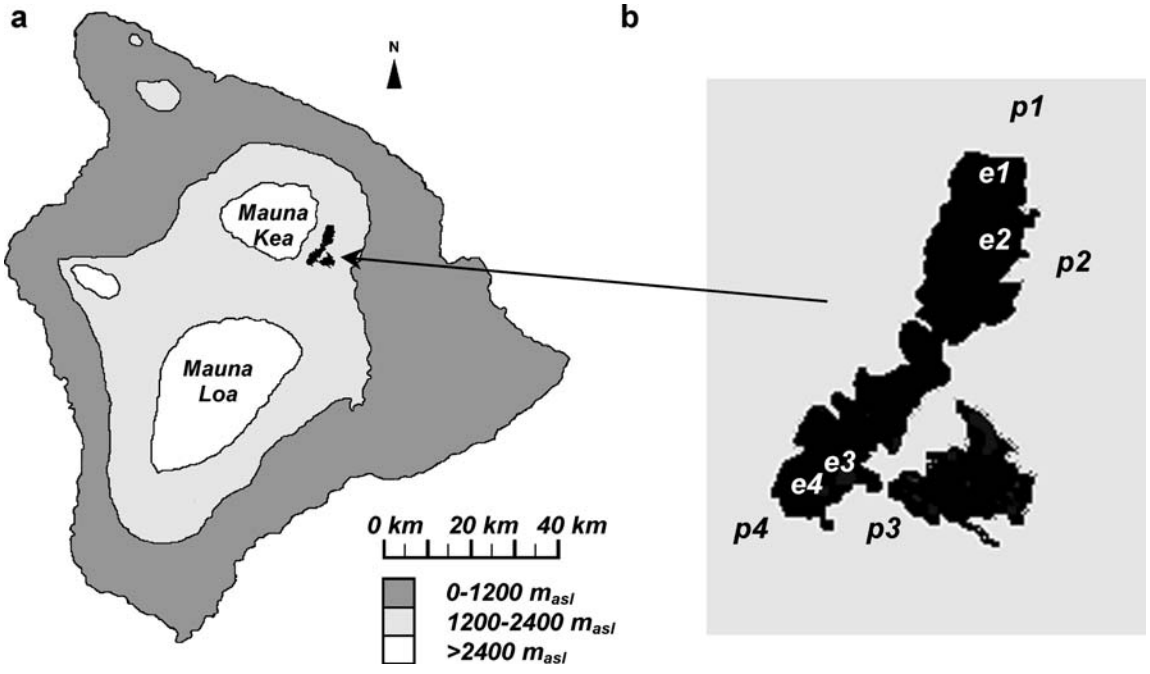

Fig. 1 **a** View of the size and distribution of gorse infestation (*black arrow*) on the southeastern slope of Mauna Kea on the Island of Hawaii. **b** Enlargement of the infestation (*arrow*) with designations showing the sampling transects for the epicenter (*e1*, *e2*, *e3*, *e4*) and corresponding periphery (*p1*, *p2*, *p3*, *p4*) zones

inside the infestation. The corresponding periphery-zone transects were established outside of the infestation, within 200 m of the infestation perimeter.

Soil analysis

Soil samples were collected 30 cm from the base of the selected mature gorse plants along each transect for the epicenter and periphery zones. For each sampling point, three core samples were collected from the top 10 cm of soil (5 cm diameter). In the periphery zones, corresponding soil samples were also collected, 2 m from the sampled gorse plants, as representative periphery samples collected within a kikuyu grass rhizosphere. Kikuyu grass rhizosphere samples could not be collected within the epicenter zones due to adjacent mature gorse plants within 1 m of the sampled gorse plant. Samples from all eight points (n=24) were bulked for further analyses. Soil pH was measured from 1:1 soil/water slurry equilibrated for 30 min (Hue and Evans 1986). The Mehlich III soil extraction procedure was used to extract P, K, Ca, Mg, Mn, Zn, B, Cu, and Fe (Mehlich 1984). Extraction of Al was performed with a 1:10 dilution of a 5-g soil sample in 1 M KCl, shaken for 30 min. All nutrients were measured with an inductively coupled plasma spectrometer (Atomscan 16, Thermo Electron Corp., California, USA).

Plant analysis

Three stem tissue samples measuring 10 cm in length were collected from the most apical portions of all eight gorse plants along each transect. Corresponding kikuyu grass stolon samples were collected within 1 m of the sampled gorse plant. These samples were collected 10 cm from the most apical portions of the stolon. Tissue samples from each transect (n=24) were bulked for further analyses. All analyses were performed at the Agricultural Diagnostic Service Center, University of Hawaii. Bulk samples were oven-dried at 70°C, then ground to pass a 0.4-mm sieve. A 0.5-g sample was ashed at 500°C in a muffle furnace and dissolved in 25 ml of 1 M HCl. The plant Al, P, K, Ca, Mg, Mn, Fe, Zn, and Cu were measured with an inductively coupled plasma spectrophotometer (Atomscan 16; Thermo Electron Corp.). Plant tissue samples were submitted to the Stable Isotope Facility at the University of California, Davis, where total N content and $^{15}N/^{14}N$ isotope ratios were determined, using an automated nitrogen analyzer, coupled to an isotope ratio mass spectrometer (Europa 20/20; SerCon Ltd., UK). Results from the analyses were used to calculate the natural abundance of ^{15}N ($\delta^{15}N$) expressed as ‰ using the equation $\delta^{15}N = [(^{15}N/^{14}N_{\text{sample}})/(^{15}N/^{14}N_{\text{standard}}) - 1]$. The $^{15}N/^{14}N$ ratio for atmospheric N_2 has little variation throughout the world and is designated as the standard reference point in this equation (Mariotti 1983).

Isolation and authentication of gorse bradyrhizobia

Nodules were collected from the root systems of all eight sample gorse plants along each transect for the first three experimental units. Nodules were also collected from 12-year-old transplanted koa trees along a single transect within the Hakalau Forest National Wildlife Refuge, adjacent to the gorse infestation. The transect was within 300 m of the infestation and sampled koa trees were separated at least 100 m from each other. Bacteria were isolated from surface-sterilized nodules and cultured on YEM agar media using the techniques described by Somasagaran and Hoben (1994). The *Bradyrhizobium* isolates were authenticated for nodulation by inoculating siratro and koa seedlings using similar techniques described by Somasagaran and Hoben (1994).

Bacterial characterization

Four gorse isolates were randomly selected from each epicenter and periphery transects in the first three experimental units (n=24). Two isolates were randomly selected from each of the ten koa trees along the adjacent transect described above (n=20). DNA extractions from these isolates were performed using the techniques described by Sritharan and Barker (1991). Fragments of 16S rDNA were generated from representative isolates using the universal eubacterial primers 27f and 1492r (Lane 1991). Polymerase chain reactions (PCR) were carried out in 50-µl volumes with 2 µl DNA supernatant, 1× buffer, 2.0 mM $MgCl_2$, 200 µM for each dNTP, 20 pmol of each primer, and 2 units of *Taq* polymerase. PCR was performed with a 96-well block thermocycler (GeneAmp 2700, Applied Biosystems, California, USA) with the following parameters: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 2 min, followed by a final extension of 72°C for 10 min. The amplified products (10 µl) were digested with the restriction endonuclease tandems *Hin*fI/*Msp*I and *Hha*I/*Rsa*I (New England Biolabs, Massachusetts, UAS) in 15-µl reactions according to the manufacturer's instructions. Ten microliters of each reaction was loaded onto 10-cm-long, 2% agarose gels, and electrophoretically resolved at room temperature in 1× Tris acetate–EDTA buffer for 2 h at 4 V cm^{-1}. Gels were visualized by ethidium bromide staining and UV excitation. Genomic fingerprints were also generated by box-PCR. The BOXA1R primer, which is directed against repetitive sequences in the bacterial genome, can generate 10–20 band fingerprints (Louws et al. 1999). Box-PCR was carried out in 12-µl volumes with 1 µl DNA supernatant. Reaction mixtures and cycling programs have been previously described (Louws and Cuppels 2001) and were also performed using a 96-well thermocycler (GeneAmp 2700; Applied Biosystems, California, USA). Ten microliters of each reaction was loaded onto 10-cm-long, 2%

agarose gels, and electrophoretically resolved at room temperature in 1× Tris acetate–EDTA buffer for 4 h at 4 V cm^{-1}. Gels were visualized by ethidium bromide staining and UV excitation.

Calculations of diversity

Fingerprints of 16S rDNA restriction fragments and genomic fingerprints generated by box-PCR were compared visually to identify unique and matching types. Discrimination was assessed by calculating Simpson's index $D=1-[\sum n_i(n_i-1)]/N(N-1)$, as described by Hunter and Gaston (1988), where n_i is number of the ith type, and N is the total number of types in the population. The index $(1-D)$ represents the probability that two individuals randomly selected from a sample will be different types.

MPN plant inoculation assay of rhizosphere soils

The numbers of rhizobia present in all epicenter and periphery soils were estimated via the most-probable-number (MPN) assay (Vincent 1970). Ten grams of fresh soil were added to 90 ml of sterile dH_2O, and tenfold dilutions were performed five times. All dilutions were inoculated on four 10-day-old siratro (*Macroptilium atropurpureum* cv. Aztec) seedlings, which were germinated from surface-sterilized seeds, grown in sterile vermiculite, and maintained with N-free plant nutrient solution (Somasagaran and Hoben 1994). Positive/negative evaluations of nodulation were made 4 weeks later and extrapolated to estimation tables.

Statistical analyses

A two-way analysis of variance (ANOVA) was performed for soil and plant data presented in Tables 1 and 2 and Fig. 2, with the experimental units and zone transects serving as sources of variation. The software program MSTAT-C (East Lansing, MI) was used for all statistical analyses.

Table 1 pH, total N, and Mehlich-III extractable nutrient concentrations of surface soil samples (0–10 cm) taken in the gorse-infested epicenter (e), and within the rhizosphere of a gorse pioneer and kikuyu grass of the periphery zone (p)

	pH	%N	P	K	Ca	Mg	Al	Mn	Zn	Cu	Fe
			(mg kg^{-1})								
Gorse (e)	5.3	1.3	18	110	1,064	188	41	51	6.7	3.6	323
Gorse (p)	5.6	0.9	22	155	1,356	320	17	57	10.6	8.6	365
Kikuyu (p)	5.8	1.2	29	237	1,555	494	20	75	13.7	4.0	361
e vs p	*	NS	NS	NS	*	**	**	*	*	NS	NS
g vs k (p)	NS	NS	NS	NS	NS	**	NS	*	NS	NS	NS

Orthogonal comparisons (df=1) between the epicenter and periphery zones and between gorse and kikuyu grass samples within the periphery zone are shown
NS Not significant
*Significant at $P\geq0.05$, **significant at $P\geq0.01$

Table 2 Nutrient concentrations of plant tissue samples taken from apical portions of gorse (g) and kikuyu grass (k) within the gorse-infested epicenter (e) and periphery zones (p)

	%N	P	K	Ca	Mg	Al	Mn	Zn	Cu	Fe
		(mg kg^{-1})								
Gorse (e)	2.41	1,100	5,467	5,533	2,400	146	90	25	6	265
Gorse (p)	3.57	1,333	8,800	4,367	2,767	132	88	30	5	257
Kikuyu (e)	1.74	1,133	6,200	3,000	2,133	1,647	160	10	3	363
Kikuyu (p)	1.45	1,467	9,933	2,533	2,867	631	216	20	2	413
g vs k	**	NS	NS	**	NS	**	**	*	*	NS
e vs p (g)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
e vs p (k)	NS	NS	NS	NS	NS	*	NS	NS	NS	NS

Orthogonal comparisons (df=1) between gorse and kikuyu grass and between the epicenter and periphery zones for each plant tissue are shown
NS Not significant
*Significant at $P\geq0.05$, **significant at $P\geq0.01$

Data showing significance for nonadditivity were log-transformed and reanalyzed. Regression analyses were performed for soil KCl-extractable Al vs soil pH, and plant $\delta^{15}N$ vs soil %N.

Results

Soil nutritional characteristics of the gorse-infested epicenter and adjacent periphery zones

To determine how gorse infestation influenced the soil environment of this volcanic landscape, we compared the soil nutritional contents between the gorse-infested epicenters and the adjacent uninfested peripheries. The soils within the epicenters of the gorse infestation were significantly more acidic than the soils within the uninfested periphery (Table 1). The epicenter soils had twofold higher levels of KCl-extractable Al than the periphery soils. There was a highly significant inverse linear relationship between the extractable Al and soil pH for all data points (r^2=0.55, $P\leq0.01$).

The gorse-infested epicenter soils had significantly lower concentrations of extractable Ca, Mg, Mn, and Zn (Table 1). The levels of extractable K and Fe were also lower in the epicenter soils, although not significant at $P\leq0.05$ (P=0.11 and P=0.06, respectively). There were no significant differences in the soil concentrations of N and P between the epicenter and periphery zones. The differences in Ca, Mg, and Al resulted in Ca:Al molar charge ratios that were almost fourfold higher in the periphery soils than in the epicenter soils, whereas the Mg:Al molar charge ratios were more than fivefold higher in the periphery soils (Fig. 2a). There were no significant differences in these ratios between the gorse and kikuyu grass rhizosphere soils of the periphery zones.

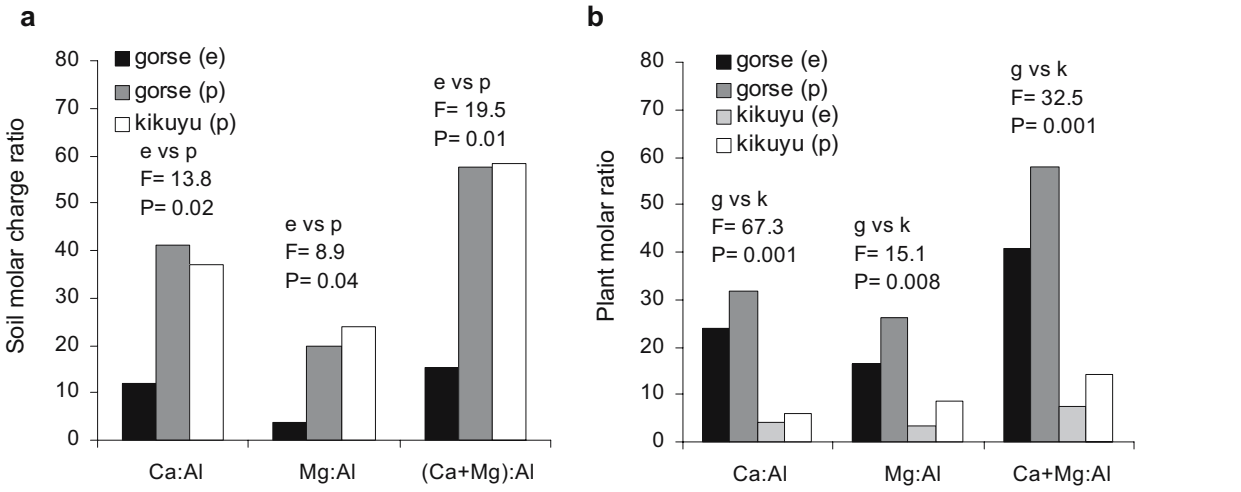

Fig. 2 **a** Soil molar charge ratios of the extractable cations Ca, Mg, and (Ca+Mg) to Al measured from soil samples taken within the rhizospheres of gorse-infested epicenters, and within the rhizospheres of gorse and kikuyu grass in the periphery zones. *F* and *P* values were generated by orthogonal comparisons (*df*=1) between the gorse-infested epicenter (*e*) and periphery (*p*) zones in a two-way ANOVA (*df*=11). **b** Plant tissue molar ratios of the cations Ca, Mg, and Ca+Mg to Al measured from gorse (*g*) and kikuyu grass (*k*) taken in the gorse-infested epicenter and periphery zones. *F* and *P* values were generated by orthogonal comparisons (*df*=1) between gorse and kikuyu grass in a two-way ANOVA (*df*=11)

Nutritional characteristics of gorse apical stem and kikuyu grass stolon tissues within the gorse-infested epicenter and adjacent periphery zones

Gorse plants accumulated significantly higher levels of Ca, Zn and Cu than kikuyu grass (Table 2). In contrast, gorse plants accumulated significantly less Al and Mn than kikuyu grass. Furthermore, Al accumulation in kikuyu grass was significantly greater in the epicenter than in the periphery zone ($P \leq 0.05$). Ca:Al molar ratios were more than fivefold higher in gorse tissues than kikuyu grass, whereas the Mg:Al molar ratios were more than threefold higher (Fig. 2b). Neither gorse nor kikuyu grass differed in these molar ratios when compared between the epicenter and the periphery zones.

Contributions of symbiotic nitrogen fixation to gorse infestation

All gorse plants, including young seedlings less than 6 months old, were nodulated on surface roots within the crown of the taproot. The nodules of mature plants measured 1–2 cm in length and had an indeterminate growth habit with multiple apices. The individual apices of the nodules were uniform in size, ranging from 2 to 3 mm in diameter. Cross sections of the nodules showed the presence of leghemoglobin, suggesting that these nodules are actively fixing nitrogen. Gorse samples from both epicenter and periphery zones had significantly higher nitrogen contents than the corresponding kikuyu grass samples (Table 2). Furthermore, the $\delta^{15}N$ values for kikuyu grass had a range of 0.93 to −4.18‰, whereas gorse samples measured within a much more narrow range of 0.32 to −0.74‰, which is closer to the reference value of 0‰ designated for atmospheric nitrogen. The $\delta^{15}N$ values for kikuyu grass showed a significant inverse linear relationship (r^2=0.54, P=0.039) with %N of the soil, whereas the gorse $\delta^{15}N$ values had no significant linear relationship (r^2=0.01, P=0.81), and was therefore independent of soil N concentrations. Thus, these data indicate that symbiotically fixed atmospheric nitrogen is accumulated within the gorse plants.

Characteristics of the indigenous bradyrhizobia nodulating gorse

Two sample populations of bacteria were established from root nodules collected from gorse and koa (see Materials and methods). Sequences of 16S rDNA from representatives of gorse and koa sample populations showed high homology to many other *Bradyrhizobium* sequences. All isolated *Bradyrhizobium* for both sample populations were able to nodulate siratro, which is known to nodulate with a broad range of bradyrhizobia. More interestingly, all *Bradyrhizobium* isolates from the gorse sample population could also nodulate koa. Restriction fragment length polymorphism (RFLP) of 16S rDNA sequences from gorse

Table 3 Richness (*S*), relative proportions of the dominant types (*P*), and Simpson's index of diversity (1−*D*) based on 16S rDNA RFLP and box-PCR fingerprint types from sample populations of *Bradyrhizobium* strains isolated from gorse (*n*=24) and koa (*n*=20) in Humuula

	S	P	1−*D*
16S rDNA RFLP			
Gorse	4	0.50	0.65
Koa	3	0.60	0.54
Box-PCR			
Gorse	10	0.50	0.75
Koa	11	0.45	0.81

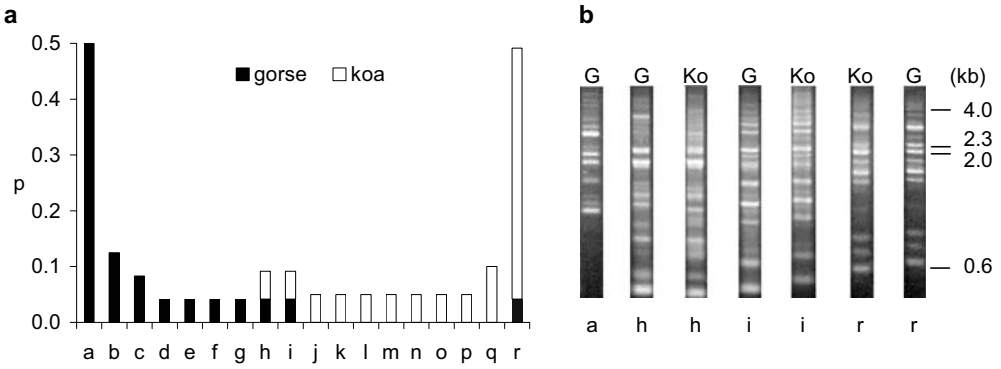

Fig. 3 **a** Proportional abundances (*p*) for box-PCR fingerprint types created from gorse-nodulating (*n*=24) and koa-nodulating (*n*=20) *Bradyrhizobium* sample populations. **b** Gel images of dominant and matching box-PCR profiles for gorse (*G*) and koa (*Ko*). *a* Dominant gorse type, *h* and *i* matching rare gorse and koa types, *r* dominant koa type and matching rare gorse type

Bradyrhizobium generated four fingerprint types, with over 37% of the gorse sample population sharing the same fingerprint patterns as the koa sample population (data not shown). Based on 16S rDNA and box-PCR fingerprints, gorse and koa sample populations have similar indices for richness and diversity (Table 3). The box-PCR results show more discrimination due to higher diversity and a greater richness of types (Fig. 3). The two sample populations can both be described as having several rare types and a single dominant type. The dominant box-PCR type for gorse was distinct from the dominant koa type. However, a rare gorse type did match with the dominant koa type. Within the gorse and koa sample populations, there were also two sets of rare types that were highly similar.

The MPN assay estimated higher densities of *Bradyrhizobium* populations within the gorse soils than within the kikuyu grass soils of the periphery zone for all four units (Table 4). The estimated populations are high for all gorse soils, with no differences estimated between gorse soils in the epicenter and periphery zones for two of the units. Three of the units show kikuyu grass soils to have low *Bradyrhizobium* densities. The high *Bradyrhizobium* population in the kikuyu grass of unit 1 may be due to the adjacent mature koa forest that is harboring bradyrhizobia within the vicinity.

Discussion

Gorse infestation in Hawaii is confined to the volcanic ash-derived soils of Maui and Hawaii. The gorse-infested soils of Humuula are derived from the volcanic ash of Mauna Kea. These soils of the Andisol order are nutritionally deficient as implied by the "dystric" designation. These volcanic soils are productive, however, when the fertility is managed (Shoji et al. 1993). The amorphic descriptor indicates that these soils have high allophane content with little crystalline structure. Allophanes are variably charged and highly pH-dependent with a wide cation exchange capacity (CEC) ranging from 50 mmol (+) kg^{-1} under acid conditions to >1,000 mmol (+) kg^{-1} near neutrality (Bohn et al. 2001). Gorse infestations in New Zealand have been documented on similar low-fertility soils of volcanic origin (Parker 1984).

Gorse is a calcifuge shrub that acidifies the surrounding soils and grows optimally at soil pH 4.5–5.0, although it is generally considered to be sensitive to alkaline soil conditions (Grubb et al. 1969). Gorse is considered to be more tolerant to soil acidity than most legumes (Hill 1949). Soil acidification occurring within a gorse infestation may be the result of nutrient-cation immobilization within the woody aboveground biomass (Egunjobi 1969, 1971; Grubb and Suter 1971). Egunjobi (1971) described the cycling of basic nutrients as an important ecological process of gorse. This explanation is consistent with descriptions in acidification of other volcanic soils occupied by different woody vegetations (Ugolini and Dahlgren 2002). In Humuula, Ca, Mg, Mn, and Zn were depleted from the acidified soils within the gorse-infested epicenter. Calcifuge plants are known to exude organic acids in the process of nutrient acquisition (Ström 1997). Organic acid extrusion from a legume root system has also been connected to active N_2 fixation (Raven et al. 1990). In the case of Humuula, basic nutrient depletion may be the direct result of active uptake and immobilization by gorse, which exceeds mineralization back into the soil. The observations in Humuula suggest that acidification by gorse may reduce the CEC of these pH-dependent soils.

The epicenter soils have significantly higher levels of extractable Al. The inverse linear relationship of extractable Al to soil pH shows that bioavailable forms of Al are more abundant under acidic soil conditions (Ritchie 1989). Al toxicity is based on both the physiology of the plant and properties of the soil (e.g., soil pH, levels of Ca, soluble organic acids). Wide variations in these factors within an ecosystem make it difficult to assign a threshold for Al toxicity (Adams 1984). A clear assessment of Al toxicity due to the increase in extractable Al caused by gorse in-

Table 4 Most probable number (MPN) plant infection assay to enumerate *Bradyrhizobium* soil populations collected within epicenter (e) and periphery (p) zones for each of the experimental units

	Bradyrhizobium (cells g^{-1}soil)			
	Unit 1	Unit 2	Unit 3	Unit 4
Gorse (e)	690,000	690,000	100,000	>700,000
Gorse (p)	690,000	690,000	3,100	340,000
Kikuyu (p)	100,000	310	<1	170

A range factor of 3.8 provides 95% fiducial limits (Cochran 1950)

festation was not undertaken in this study. However, Awad et al. (1976) has reported that Al toxicity to kikuyu grass occurred when foliar Al concentrations exceeded 90 μg g^{-1}, a level well below our reported values.

Aluminum can displace Ca and Mg from soil exchange sites and within root cortical apoplasts, resulting in a low uptake of Ca and Mg by the plant (Jentschke et al. 1991). Low Ca:Al and Mg:Al soil ratios have served as valuable indicators for forest ecosystems under stress (Cronan and Grigal 1995; Sverdrup et al. 1992). The low Ca/Al and Mg/Al molar charge ratios of the infested soil may be an indication of environmental stresses incurred on the landscape by the long-term growth and activity of gorse. Three decades of loblolly (*Pinus taeda*) pine forest development in southeastern USA have led to significant depletions in Ca and Mg along with simultaneous increases in exchangeable acidity (H^+ and Al^{3+} ions) (Richter et al. 1994). Gorse stands tend to live as even-aged cohorts that can live for up to 30 years (Lee et al. 1986; Ivens 1983). Successional development of this gorse infestation may have led to the current state of low fertility and declining Ca:Al and Mg:Al soil molar charge ratios.

The gorse and kikuyu grass Ca:Al molar ratios that were measured in Humuula are within the reported ranges for tropical pasture legumes as well as temperate forest trees (Cronan and Grigal 1995; Poolpipatana and Hue 1994). The tissue ratios in kikuyu grass in this study indicate that there may be an antagonism from the bioavailable Al to basic cation uptake. Excess Al has previously been demonstrated to reduce basic cation uptake by kikuyu grass (Huett and Menary 1980). Kikuyu grass that exhibited reduced growth in the study by Awad et al. (1976) had Ca:Al and Mg:Al plant molar ratios similar to those measured for kikuyu grass in this study. The low levels of Al measured within the stem tissues of gorse suggest that the plant has a mechanism of Al exclusion, thereby maintaining relatively high basic cation:Al molar ratios.

It is generally recognized that many of the world's environmental weeds are symbiotic nitrogen-fixing legumes, including gorse (Richardson et al. 2000). Based on nodulation of the root systems, higher N levels in the stem tissues, corresponding $\delta^{15}N$ values approaching 0‰, and interactions with the indigenous bradyrhizobia in the soil, it is apparent that symbiotic nitrogen fixation is an important function for gorse plants established in Humuula. Since volcanic ash-derived soils on the Island of Hawaii are typically N-deficient (Vitousek and Farrington 1997; Raich et al. 1996), gorse, being a legume, has an advantage over other plant species in Humuula. Gorse nodulation is perennial (Pate 1961). Furthermore, gorse grows year-round in this mild climate of Humuula, suggesting that nitrogen fixation is also year-round. It is worth noting that the pioneer gorse plants within the periphery zone consistently had higher levels of N than gorse plants in the infested epicenter, which may be the result of cohort competition taking place within the infestation epicenter. Incidentally, kikuyu grass should contain up to 4% N under optimal growing conditions (Mears 1970). The N contents of kikuyu grass measured within this infested site were much lower, suggesting a deficiency.

The successful invasion of gorse in Hawaii may have been expedited by compatible symbiotic interactions with the indigenous bradyrhizobia in the soil. Legumes will often fail to colonize a local habitat where the symbiotic partners are scarce (Parker 2001). Bradyrhizobia are symbionts with broad host range and are ubiquitous throughout the soils of Hawaii, particularly where koa forests are established (Nakao and Kitayama 1996; Woomer et al. 1988). The data showing gorse *Bradyrhizobium* cross nodulating koa and also matching genetic fingerprint types suggest that gorse and koa *Bradyrhizobium* isolated from Humuula are highly related. Thus, gorse may have adapted to the bradyrhizobia originally associated with koa. This report of high *Bradyrhizobium* populations associated with gorse is consistent with another report describing the long-term establishment of legumes being influential to maintaining soil microsymbiont populations (Woomer et al. 1988). Perennial legumes that are established long-term within a natural environment are also known to select for specific *Bradyrhizobium* (Parker 1995; Wilkinsen et al. 1996). Gorse has been established in Hawaii for almost a century (Motooka et al. 2003), while koa has been established long enough to evolve into an endemic species. The box-PCR method can be used to identify diversity of *Bradyrhizobium* at the subspecies level (Vinuesa et al. 1998). Our data suggest that the exotic gorse and endemic koa interact with the same *Bradyrhizobium* population, but have selected for distinct dominant box-PCR types that may have unique fitness.

Conclusion

This report describes biotic and abiotic characteristics associated with gorse infestation in a high-elevation, volcanic landscape of Hawaii. This habitat appears to be suitable for this invasive weed to become the dominant vegetation of the landscape. Humuula and other gorse-infested landscapes share a common history of logging and overgrazing subsequent to weed invasion. Thus, the site-specific information describing the infestation in Humuula may also be a common feature for other gorse infestations.

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