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Invasion of Hawaiian rainforests by an introduced amphibian predator and N_2-fixing tree increases soil N_2O emissions

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Citation: Hall, S. J., D. P. Huber, and R. F. Hughes. 2018. Invasion of Hawaiian rainforests by an introduced amphibian predator and N_2-fixing tree increases soil N_2O emissions. Ecosphere 9(9):e02416. 10.1002/ecs2.2416

Abstract. Invasions of introduced species have homogenized ecological communities worldwide, leading to losses of native species and the services they provide. Some of these invaders substantially alter nutrient cycling, which changes conditions for all other organisms, but less is known about the potential influence of these species on nitrogen (N) trace gas emissions that affect atmospheric processes. We used a natural experiment to explore whether the establishment of an introduced nitrogen (N) fixing tree (*Falcataria moluccana*) and recent invasion of an amphibian predator, the Caribbean tree frog (*Eleutherodactylus coqui*), into native Hawaiian rainforests have affected soil emissions of nitrous oxide (N_2O) and nitric oxide (NO), two atmospherically important trace gases produced by soil microorganisms. Soil N_2O and NO emissions and rates of soil N cycling were significantly higher in *F. moluccana*-dominated stands compared to native *Metrosideros polymorpha* (Ohi'a) stands. Additionally, invasion of *E. coqui* frogs moderately increased soil N_2O emissions, primarily in non-native *F. moluccana* forests where soil N availability was already elevated. N_2O emissions were positively and significantly related to net potential N mineralization, and total N_2O+NO fluxes increased with soil nitrate (NO_3^-) concentration and rates of nitrification. Previous work in these Hawaiian rainforest sites has shown that *F. moluccana* substantially increases N availability by increasing ecosystem N supply compared to uninvaded stands, and *E. coqui* accelerates N availability and litter decomposition, although moderately, due to enhanced fluxes of nutrient-rich waste products. Here, we show that acceleration of nutrient cycling by introduced species can also alter biosphere–atmosphere exchange of N-oxides.

Key words: Albizia; *Eleutherodactylus coqui; Falcataria moluccana; Metrosideros polymorpha;* N trace gas; nitric oxide; nitrification; nitrous oxide; N-oxide; predator; soil; trophic.

Received 10 July 2018; accepted 16 July 2018. Corresponding Editor: Debra P. C. Peters.

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INTRODUCTION

Non-native species invasions have altered biotic communities worldwide, leading to native species declines and changes in ecosystem structure (Asner et al. 2008*a*, Doherty et al. 2016, Vanbergen et al. 2018). Some invasive species also affect ecosystem processes such as primary productivity and nutrient cycling, which influence ecosystem services and stability (Ehrenfeld 2003, Vilà and Hulme 2017). Ecosystem-level consequences of biotic introductions result when traits of non-native invasive species are functionally distinct from the native biota, particularly those traits that alter the magnitude or timing of resource supply, climate, habitat structure, disturbance regimes, or food webs (Chapin et al. 2000, Ehrenfeld 2003, Lee et al. 2017). For example, in forests where nitrogen (N) limits primary production, non-native N_2-fixing plants can significantly speed

nutrient cycling and ecosystem development (Vitousek and Walker 1989). Introduced predators have also been shown to cause significant changes to native biotic communities, particularly on islands where functionally similar species are absent, with cascading consequences for soils and plant growth (Fukami et al. 2007).

Although not as well studied as local community and ecosystem-level effects, introduced species can also have consequences well beyond their physical locations by altering regional atmospheric gas fluxes that affect climate and air quality (Hall and Asner 2007, Talluto and Suding 2008, Hickman et al. 2010, Qiu 2015). Nitrous oxide (N_2O) is a long-lived, potent greenhouse gas with a global warming potential ~265 times that of CO_2, and in the stratosphere, it can catalyze the destruction of UV-absorbing ozone (Ravishankara et al. 2009, IPCC 2014). Nitric oxide (NO) is highly reactive in the troposphere, and combined with NO_2 ($NO + NO_2 = NO_x$), it contributes significantly to nitrogen (N) deposition, smog, and other regional air quality problems (Crutzen 1979, Chameides et al. 1992). N_2O and NO are produced in soil primarily through microbial processes that oxidize or reduce N compounds, including the aerobic process of nitrification (oxidation of ammonium [NH_4^+] or organic N to nitrite and nitrate [NO_3^-]) and the anaerobic process of denitrification (reduction of NO_3^- to NO, N_2O, and dinitrogen [N_2]). N availability is the primary driver of soil N_2O and NO emissions as it fuels the flow through the linked nitrification and denitrification pipe as described in the hole-in-the-pipe (HIP) model (Firestone and Davidson 1989). Additionally, soil moisture and soil carbon (C) assert secondary controls over soil N-oxide emissions by reducing oxygen availability and providing electron donors for denitrification (Davidson et al. 2000). Based on these mechanisms, we expect that any introduced non-native species that significantly alters rates of soil N cycling would affect total emissions of N_2O and NO from soil and that soil moisture would increase the ratio of N_2O to NO, possibly by favoring denitrification processes over nitrification.

Despite the nearly 17,000 introduced species currently established in ecosystems globally (Seebens et al. 2017), few studies have evaluated the influence of non-natives on soil N_2O or NO emissions (Qiu 2015). Only six non-native, introduced plant species have been examined for their effects on N-oxide emissions in field settings. Of these, species with the largest effects were those that significantly increased the speed of N cycling, likely through N-fixation (e.g., *Morella faya*/fire tree, Hall and Asner 2007, and *Pueraria montana*/kudzu, Hickman et al. 2010); altered the timing of N mineralization through more frequent wet–dry cycles (e.g., *Bromus tectorum*/cheatgrass, Norton et al. 2008); or presumably increased plant NO_3^- demand and rates of N_2O consumption, thereby reducing the availability of electron acceptors for denitrification (e.g., *Trapa natans*/water chestnut, Tall et al. 2011, and *Spartina alterniflora*/smooth cordgrass, Yuan et al. 2015).

Although a few studies have suggested that exotic earthworms could have field-level effects on N_2O (data from mesocosms, or inferred from denitrification; Kim et al. 2015, Fugere et al. 2017), to date no studies have evaluated the effects of non-native animals on N_2O or NO emissions in field settings. Animals can produce N-oxides through the activity of denitrifying organisms in their guts or in biofilms associated with their shells (e.g., aquatic macrofauna, earthworms, and termites; Drake and Horn 2007, Stief et al. 2009, Heisterkamp et al. 2013, 2016, Lubbers et al. 2013, Brauman et al. 2015). Additionally, animals can influence soil N-oxide fluxes by directly modifying soil properties that influence soil nitrifying or denitrifying microbes (e.g., aeration, N availability) or by indirectly modifying soil properties through changes in community composition or dynamics.

Non-native predators can also significantly alter ecosystem functioning (Wardle et al. 2011, Tronstad et al. 2015). Extensive evidence indicates that terrestrial and aquatic predators can indirectly affect carbon (C) and N cycling by altering the abundance or behavior of species at lower trophic levels (Dobson et al. 2006, Schmitz et al. 2010, Strickland et al. 2013). For example, predation on detritivores can slow litter decomposition (Ruetz et al. 2002, Wu et al. 2014). Alternatively, predation can increase soil fertility when predators release bioavailable nutrients through excretion that would have otherwise been immobilized by prey species (Clarholm 1985, Ngai and Srivastava 2006, Hinchliffe et al. 2017). For example, invasion of Hawaiian rainforests by the predatory

Caribbean tree frog, *Eleutherodactylus coqui*, increases rates of litter decomposition and nutrient cycling due to excretion of labile N following its consumption of detritivorous leaf litter arthropods (Sin et al. 2008).

As the most isolated archipelago on Earth, the Hawaiian Islands have served as an important natural laboratory for studies on non-native biotic invasions (Mooney and Drake 1986, Vitousek et al. 1987, Mack and D'Antonio 1998). *Falcataria moluccana* (Albizia), among the world's fastest-growing tree species and an N-fixer, was intentionally introduced to Hawai'i in 1917 from its native range across Indonesia, Papua New Guinea, and the Solomon Islands. Following its introduction and throughout the mid-1900s, populations of *F. moluccana* were purposefully established across many of Hawaii's forest reserves, and it has since naturalized into lowland Hawaiian rainforest ecosystems that were formerly dominated by the native tree, *Metrosideros polymorpha* (Ohi'a). In eastern Hawai'i, as expected based on its relationship with N-fixing microorganisms, *F. moluccana* has dramatically increased rates of litterfall (by eightfold), soil bacterial: fungal ratios, N and phosphorus (P) cycling (by 55- and 28-fold, respectively), NO_3^- concentrations in streams (twofold), and litter decomposition (2- to 10-fold) compared to native, N-limited *M. polymorpha* forests (Hughes and Denslow 2005, Allison et al. 2006, Hughes and Uowolo 2006, Wiegner et al. 2013). *F. moluccana* currently occupies approximately 14,000–16,000 ha of young, N-poor soils on the eastern side of Hawaii Island and is also widespread across other islands in the Hawaiian Archipelago (Watson 2017, Niemiec et al. 2018).

More recently, the insectivorous frog, *E. coqui,* has invaded both native *M. polymorpha* and non-native *F. moluccana* forests in Hawai'i, where no native, terrestrial amphibians or reptiles occur. *E. coqui* was inadvertently introduced to Hawai'i from Puerto Rico by the nursery trade during the late 1980s, and it is now present across most of the Hawaiian Islands (USGS 2018). Amphibian species are in decline worldwide due to multiple stressors, including infection by the pathogenic fungus, *Batrachochytrium dendrobatidis* (Bd; Collins and Crump 2009). Although Bd also infects *E. coqui* (Beard and O'Neill 2005), population densities are higher in Hawaiian rainforests compared to their native habitat, averaging 2200–50,000 frogs per ha on Hawai'i Island (Beard et al. 2008). These predatory frogs eat detritus-consuming arthropods from leaf litter, leading to significant changes in invertebrate community composition (Beard 2007, Choi and Beard 2012). *E. coqui* invasion also moderately increases rates of litter decomposition (by 3%) and nutrient cycling (47% and 8% increase of NH_4^+ and phosphorus in leaf washes, respectively), but this outcome is primarily due to increased excretory nutrient fluxes into the litter pool rather than through alterations in invertebrate (prey) abundance (Sin et al. 2008).

In this study, we build on a body of ecosystem-level research from a natural experiment on Hawai'i Island (hereafter referred to as Hawai'i) to explore effects of two dominant non-native species on N_2O and NO_x emissions from soil. Around 2006, monodominant stands of native *Metrosideros polymorpha* and non-native *Falcataria moluccana* in eastern Hawai'i were invaded by the non-native frog, *Eleutherodactylus coqui.* Extensive research at this time was conducted on these particular sites to evaluate the impacts of these non-native species invasions on ecosystem processes (Allison et al. 2006, Hughes and Uowolo 2006, Beard et al. 2008, McGuire 2008, Norman 2008, Sin et al. 2008, Warrington 2008, Tuttle et al. 2009, Choi and Beard 2012, among others). Here, we add to this published work by evaluating the separate and combined effects of *E. coqui* and *F. moluccana* invasion on soil N-oxide emissions.

We expected that invasion of native *M. polymorpha* forests by *F. moluccana* would increase soil emissions of N_2O and NO and rates of nitrification due to increased inorganic N supply. We based these expectations on former work in these study sites and soil N-oxide outcomes of other N-fixing tree invasions in N-limited forests of Hawai'i (Hughes and Denslow 2005, Hughes and Uowolo 2006, Hall and Asner 2007). We also expected invasion of native *M. polymorpha* forests by *E. coqui* to increase N-oxide emissions due to *E. coqui*-driven acceleration of nutrient cycling and litter decomposition (Sin et al. 2008). In contrast, we expected *E. coqui* invasion of *F. moluccana*-invaded forests to have no significant effect on soil N_2O and NO flux, likely due to relatively small changes in litter chemistry, decomposition rates, and invertebrate community composition

following *E. coqui* invasion relative to the large changes in N cycling that occur with *F. moluccana* invasion (Hughes and Denslow 2005, Hughes and Uowolo 2006, Norman 2008, Tuttle et al. 2009).

Methods

Site description and experimental design

In June–July of 2006, we measured the separate and combined effects of *E. coqui* and *F. moluccana* on soil N trace gas emissions, soil properties, and soil N cycling from young, lowland Hawaiian rainforests on Hawai'i. We tested our hypotheses across natural invasion fronts with a nested design, where forest stands were either dominated by the native tree, *M. polymorpha*, or by the non-native tree, *F. moluccana*, and were embedded within forest reserves that had been recently invaded—or were not yet invaded—by *E. coqui* (Fig. 1 and Table 1). Sites were located on chemically similar basaltic lava flows, including a young 214 y.o. lava flow in the Malama Ki Forest Reserve (*E. coqui* absent) and in Lava Trees State Monument (*E. coqui* present), and on older flows (200–750 y.o.) within Nanawale (*E. coqui* present) and Keauohana (*E. coqui* absent) Forest Reserves. The younger flow is composed of pāhoehoe-type lava that is smooth and rope-like, with large cracks. The older flows are composed of 'a'ā-type lava rocks that are sharp and jagged and thus more susceptible to weathering. All sites were located between 30 m and 278 m in elevation with mean annual temperature of 22–23°C and annual rainfall ranging from 2340 to 3150 mm/yr (Wolfe and Morris 1996, Giambelluca et al. 2013, 2014). Sites were relatively flat in slope and had not been directly disturbed by human activity.

Metrosideros polymorpha sites were dominated by *M. polymorpha* in the overstory except those in the Nanawale Forest Reserve, where the overstory was composed of *M. polymorpha* mixed with a non-native, non-N-fixing tree species,

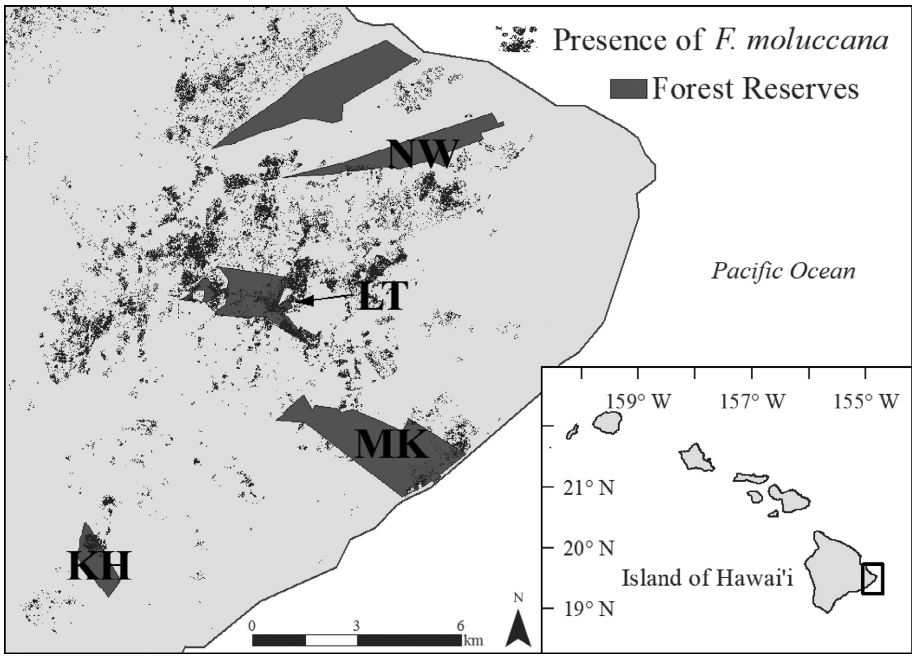

Fig. 1. Map of sampling locations in young rainforests on the eastern side of the Big Island of Hawaii. Site codes represent Nanawale Forest Reserve (NW) and Lava Trees State Monument (LT), both of which were invaded by the frog *Eleutherodactylus coqui*. Malama Ki Forest Reserve (MK) and Keauohana Forest Reserve (KH) had not yet been invaded by *E. coqui* at the time of this study. Within each of these forest reserves, we measured N-oxide fluxes and soil properties from forest stands that were dominated by either the native tree, *Metrosideros polymorpha*, or the non-native N-fixing tree, *Falcataria moluccana*. Data on *F. moluccana* distribution are from Julie Gaertner, cartographer, Big Island Invasive Species Council.

Table 1. Description of Hawaiian rainforest sites used in this study.

Location	Site	*E. coqui* density (Number/ha)†	Dominant tree species	Flow age (YBP)	Lava form	Elevation (m)	MAP (mm)	Overstory composition
MK	MK-M	0	*M. polymorpha*	214	Pāhoehoe	30	2340	*M. polymorpha*
MK	MK-F	0	*F. moluccana*	214	Pāhoehoe	32	2370	*F. moluccana*
LT	LT-M	7450 ± 745	*M. polymorpha*	214	Broken pāhoehoe	181	3140	*M. polymorpha*
LT	LT-F	7976 ± 1701	*F. moluccana*	214	Broken pāhoehoe	184	3150	*F. moluccana*
KH	KH-M	0	*M. polymorpha*	200–400	ʻAʻā	260	2860	*M. polymorpha*
KH	KH-F	0	*F. moluccana*	200–400	ʻAʻā	278	2900	*F. moluccana*
NW	NW-M	8019 ± 1238	*M. polymorpha*	400–750	ʻAʻā	85	3150	*M. polymorpha-P. cattleianum*
NW	NW-F	7541 ± 2645	*F. moluccana*	400–750	ʻAʻā	85	3150	*F. moluccana*

Note: † Adults + subadults.

Psidium cattleianum (strawberry guava). The understory of *M. polymorpha* forests consisted of non-N-fixing native and introduced species, including mixed fern, grass, and non-native bamboo; *P. cattleianum*; or another non-native shrub, *Clidemia hirta*. *F. moluccana* sites were most likely invaded within the last 30 yr (Hughes and Uowolo 2006) and were dominated by large (~10–30 m height), broad-canopy *F. moluccana* trees in the overstory with *P. cattleianum* and/or *C. hirta* in the understory.

Earthworms have been shown to increase soil N_2O emissions through their effects on soil nutrient cycling as well as denitrification processes that occur in their guts, although most evidence of earthworm-N_2O linkages comes from agricultural rather than forest ecosystems (Lubbers et al. 2013). Non-native earthworms also occur in Hawai'i and in our study area. However, earthworm abundance was significantly lower in nearby forest plots that had been invaded by *E. coqui* compared to plots where *E. coqui* had not yet invaded (Norman 2008). Furthermore, other work on non-native N-fixing trees in Hawai'i shows that earthworm density is threefold higher under the non-native N-fixer *Myrica faya* compared to *M. polymorpha* (Aplet 1990) and fivefold higher under *F. moluccana* compared to the non-N-fixer *Eucalyptus saligna* (Zou 1993). Thus, earthworm abundance is not likely to contribute to elevated N-oxide flux in *E. coqui* invaded forests compared to forests where *E. coqui* is absent. In contrast, non-native earthworms likely co-occur with *F. moluccana* invasion in our study site and may contribute to patterns in N-oxides from those sites.

In 2007, the population density of *E. coqui* was estimated to be 7780 ± 1708 adults and subadults/ha in Nanawale Forest Reserve and 7713 ± 1115 adults and subadults/ha in Lava Trees State Monument, with no significant difference between *M. polymorpha* and *F. moluccana* stands (Table 1; McGuire 2008). *E. coqui* populations had spread into the study area by the early 2000s, but significant areas of forest were still unoccupied in 2006 even though they were considered suitable for *E. coqui* invasion (Bisrat et al. 2012). Control treatments to reduce *E. coqui* numbers on Hawai'i Island did not start until 2007, a year after field work for this study was completed (Beard and Pitt 2012). In our study sites, the presence or absence of *E. coqui* was verified by intensive population studies conducted in this area in 2006 (R. McGuire, *personal communication*).

The non-native, insectivorous, and cryptic Cuban greenhouse frog, *Eleutherodactylus planirostris*, has also established in eastern Hawai'i at potentially similar densities (i.e., mean density of 2400–5300 frogs/ha; Olson et al. 2012*a*), though detection of *E. planirostris* is difficult due to its quiet call. Consequently, the abundance of this species within our study area was not known (Olson et al. 2012*b*).

Soil N_2O and NO emissions

Between 29 June and 5 July, we haphazardly placed eight PVC rings (25 cm diameter) in the soil at each site between 1 m and 5 m apart from one another across an approximately 36-m^2 area to serve as anchors for the closed-top chambers used for soil N-oxide gas measurements (N-oxide = N_2O and/or NO). In selecting sites, care was taken to ensure the area was as representative as possible of the larger forest stand. All gas measurements occurred between 11 am and 5 pm to

minimize temperature differences among sites. These tropical rainforest sites receive relatively consistent rainfall throughout the year. A total of 39 cm of rain fell during June (15 cm) and July (24 cm) 2006, with no more than 5 d exhibiting rainfall <0.1 cm (Appendix S1: Fig. S1; NWS 2006).

N-oxide gases were measured using techniques described in detail by Hall and Matson (2003). In order to measure N_2O, we placed molded acrylonitrile butadiene styrene chambers over each PVC ring in the soil, and we sampled air from the chamber four times during a 30-min period. Twenty-five mL of air from each chamber were collected in nylon syringes and immediately injected into pre-evacuated, silicone-sealed 10-mL glass Wheaton vials that were fitted with inert butyl rubber stoppers. Vials were overpressured to ensure minimal gas contamination. Samples were flown to Arizona State University (ASU) under cabin pressure, and N_2O was analyzed using a gas chromatograph fitted with an electron capture detector (Varian 3800, now Agilent, Santa Clara, California, USA) that was calibrated using certified N_2O standards in the laboratory (Scott Specialty Gases, Riverside, California, USA). N_2O standards were also injected into vials and brought to the field to correct for environmental factors influencing sample concentrations. N_2O flux was calculated as the linear increase in concentration within each chamber over the 30-min sampling period.

Nitric oxide was measured in situ at each site within Teflon-lined soil chambers using a portable chemiluminescent detector fitted with a CrO_3 filter that converted all NO to NO_2 (LMA-3, Unisearch Associates, Concord, Ontario, Canada). The instrument was operated at a 1:4 ratio of chamber air and air passed over columns of anhydrous $CaSO_4$- and NaOH-coated silica to remove humidity and CO_2 interferences (Davidson et al. 1991, Matson et al. 1996, Hutchinson et al. 1999). NO_2 concentrations were estimated photochemically following reaction with Luminol II solution. Soil NO_2 fluxes were measured occasionally and were always undetectable; as a result, all chemiluminescent NO_2 measurements were assumed to be equivalent to measurement of NO. Other chemicals known to interfere with the Luminol reaction (e.g., ozone and peroxyacetyl nitrate) were not measured in this study but are known to exist at such low concentrations in the atmosphere in east Hawai'i that they are of little concern (Walega et al. 1992, Huebert et al. 1999). Standard curves were performed in the field several times a day before, during, and after gas sampling using a known concentration of NO standard gas (0.101 ppm NO, Scott-Marrin, Riverside, California, USA). Nitric oxide fluxes were calculated as the linear slope of the NO concentration in the chamber over a 4-min period, corrected for the dilution of the headspace by ambient air inflow into the chamber using the following equation (Martin et al. 2003, Venterea et al. 2003):

$$F_{NO} = \frac{dC}{dt}\frac{V}{A} + C_A\frac{Q}{A}$$

where F_{NO} is the flux of nitric oxide (ng $N{\cdot}cm^{-2}{\cdot}h^{-1}$), dC/dt is the rate of change of NO concentration (ng N/cm^3) within the chamber over time (h) determined by linear regression, V is the chamber volume corrected for the ring height (cm^3), A is the soil surface area (508 cm^2), C_A is the average NO concentration during the time interval of regression, and Q is the air flow rate through the chamber (cm^3/h).

Soil properties, soil N concentrations, and soil N transformations

On 14 July, we collected three soil samples from each site, each of which was composed of three surface soil cores (0–10 cm depth) collected 1–10 m apart from one another and composited in the field. All soil cores were held at 10°C on ice in coolers until processing to minimize microbial activity but prevent cold shock that occurs with refrigeration of tropical soil (Verchot 1999). Within 24 h of soil collection, one-half of the homogenized sample was hand-sieved (to 4 mm) to remove roots and rocks and subsequently extracted with KCl for initial and post-incubation inorganic N concentrations (NH_4^+, NO_3^-), and analyzed for pH and soil moisture. The other half was stored on ice in coolers, shipped back to ASU, hand-sieved, and processed for nitrification potential assays within 7–16 d using methods described in Hall and Matson (2003).

Extractable inorganic N pools and rates of N transformations, soil gravimetric moisture, and soil pH.—One 10 g subsample from each plot was shaken for 1 min in 50 mL 2N KCl, set aside for 18–24 h, filtered through pre-leached Whatman

#1 filters, and frozen immediately for later analysis. Another 10 g subsample was placed in a small, capped cup and placed in the dark for 7–10 d at 23°C. After the incubation, soils were extracted as described above. A third 25 g subsample was dried at 105°C for 24 h to determine gravimetric soil moisture (g H_2O/g dry soil). All frozen KCl extracts were shipped to ASU on ice and analyzed colorimetrically for NH_4^+-N and NO_3^--N at ASU using a Lachat QuikChem 8800 autoanalyzer (Loveland, Colorado, USA). Net potential N mineralization was calculated as the difference between the sum of NH_4^+ and NO_3^- concentrations before and after each incubation. Net potential nitrification was calculated as the difference between NO_3^- concentrations before and after each incubation. To measure soil pH, twenty-five mL of 0.01 mol/L $CaCl_2$ was added to 25 g soil, slurries were shaken, and pH was determined between 0.25 and 1 h after $CaCl_2$ addition using a portable pH meter (Orion Scientific, now called Thermo Fisher Scientific, Waltham, Massachussettes, USA) calibrated with certified buffers.

Nitrification potential shaken-slurry assay.—We used methods described in Hart et al. (1994) to assess rates of potential nitrification in all soils. Potential rates of nitrification estimated with this assay have been used as an index of aerobic nitrifier population size, as rates are determined under conditions of neutral pH, and in which microbes are aerated with no diffusional limitations and plentiful NH_4^+ substrate (Hart et al. 1994, Drury et al. 2007). Between 10 and 25 g of hand-sieved soil was added to flasks along with 100 mL of a solution containing 50 mmol/L $(NH_4)_2SO_4$, 0.2 mol/L K_2PO_4, 0.2 mol/L KH_2PO_4 and adjusted to pH 7.2. Blanks of solution without soil were also processed at this time. Soil slurries were shaken vigorously on a mixer to maintain aerobicity for 24 h at 23°C. At four times within this 24-h period, 5 mL aliquots of soil slurry were removed from the flasks, centrifuged, flocculated with a 0.6 mol/L solution of $MgCl_2$ + $CaCl_2$ to aid in filtration, filtered through pre-leached Whatman #42 filters, and frozen pending analysis. Extracts were analyzed colorimetrically as above for NO_3^- at ASU. Rates of potential nitrification were determined using the positive linear slope of NO_3^- concentrations in soil extracts over the 24-h period.

Statistical analyses

We used linear mixed-effects models to draw conclusions about the independent and combined effects of *E. coqui* and *F. moluccana* on soil N-oxide emissions within this nested, landscape-level natural experiment (Davies and Gray 2015). We first explored relationships among three dependent variables (N_2O, NO, and N_2O:NO ratio) and our two main predictors and their interaction, including the absence or presence of *E. coqui*, and tree species (*M. polymorpha* or *F. moluccana*). Site was modeled as a random factor (intercept only) using the restricted maximum-likelihood estimation approach to account for potential spatial autocorrelation in gas emissions between the 8 chambers within each site. All dependent variables were transformed and residuals were inspected to check linear model assumptions. For N_2O, we confirmed the *E. coqui* × tree species interaction with two post hoc, one-way mixed models (site as random) of N_2O vs. *E. coqui* in each forest type.

We assessed the effect of *E. coqui* and *F. moluccana* on soil properties and nutrient transformations using principal components factor analysis with a direct oblimin rotation on the correlation matrix of soil variables measured from samples collected at each site (N = 3 samples per site composed of three cores each, N = 24 soil samples in total). These predictor variables were transformed as necessary to meet assumptions of normality, and they included gravimetric soil moisture, pH, and all measured N pools and transformation rates. Components with eigenvalues above 1 were retained in the analysis. When interpreting the pattern matrix, a variable was said to load on a given component if the factor scores were greater than 0.6 or less than −0.6. Multivariate analysis of variance (MANOVA) was used to assess the influence of *E. coqui* and *F. moluccana* on both principal components, and Pillai's trace was used to assess significance of each component. Because PC2 was heteroskedastic, we confirmed results from the MANOVA (effect of *E. coqui* and *F. moluccana*) with univariate ANOVA for PC1 and non-parametric Mann–Whitney *U* tests for PC2.

Finally, we conducted multiple linear regression analyses between site-averaged gas emission data (N_2O, NO, N_2O+NO, or N_2O:NO ratio) and site-averaged soil data to explore effects of

site-level soil properties and nutrient transformations on soil N-oxide emissions. After correlation analysis between our predictor variables, we chose gravimetric soil moisture, net potential N mineralization, pH, soil NH_4^+, soil NO_3^-, and nitrification potential to test in the model. We excluded net nitrification, which was significantly correlated to net potential N mineralization and nitrification potential. We used the best subsets function in SPSS and information criterion approach to calculate Akaike weights for each model (Burnham et al. 2011) and selected all models with an Akaike weight of 15% or greater. All analyses were conducted in SPSS 25 (IBM Corp. 2017).

Results

Consistent with our expectations, soil N_2O and NO emissions were significantly higher in naturalized stands of the introduced N_2-fixing tree *F. moluccana* compared to native *M. polymorpha* stands (Fig. 2). Average N_2O fluxes in sites without *E. coqui* ranged from −0.4 to 0.3 ng $N{\cdot}cm^{-2}{\cdot}h^{-1}$ in native forest stands and from 0.2 to 1.5 ng $N{\cdot}cm^{-2}{\cdot}h^{-1}$ in *F. moluccana*-invaded stands. Fluxes of NO were distributed similarly to N_2O: In sites not invaded by *E. coqui*, NO was lowest (−0.1 to 4.4 ng $N{\cdot}cm^{-2}{\cdot}h^{-1}$, min–max) in *M. polymorpha* forests and highest rates (1.1–29.6 ng $N{\cdot}cm^{-2}{\cdot}h^{-1}$) in *F. moluccana* forest.

Falcataria moluccana invasion also increased soil N concentrations and rates of N cycling. In the absence of *E. coqui*, mean soil NO_3^- concentrations increased from 10 μg N/g in *M. polymorpha* stands to 61 μg N/g in *F. moluccana* stands (Table 2). Principal components analyses of soil properties and processes revealed that rates of soil N transformations and NO_3^- concentrations were significantly elevated in *F. moluccana* compared to *M. polymorpha* stands (Fig. 3a). MANOVA tests showed that tree species was significantly associated with PC1 ($P < 0.001$), which comprised nearly half of the variance in all soil properties (Table 3). Positive values along PC1 were significantly associated with rates of net potential N mineralization and nitrification, nitrification potential—a proxy for nitrifier population size—and soil NO_3^- concentration (Table 3).

Contrary to our expectations, invasion by *E. coqui* into Hawaiian rainforests increased soil

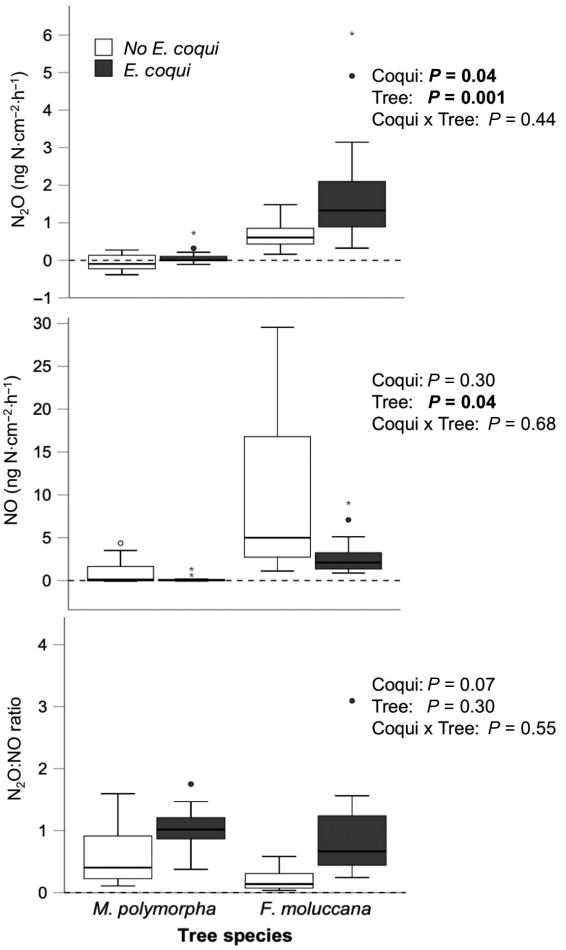

Fig. 2. Emissions of N_2O, NO, and the ratio of N_2O/NO from native and invaded Hawaiian rainforests. Statistics are significance results from linear mixed-effects model analyses.

N_2O emissions in *F. moluccana* stands (Fig. 2a), although the relative effect of *E. coqui* was substantially lower than for *F. moluccana* (Appendix S1: Tables S1 and S2). Results of the two-factor mixed model indicated a non-significant *E. coqui* × tree species interaction (Fig. 2 and Appendix S1: Table S2), but post hoc, one-way mixed models on each forest type alone showed that *E. coqui* increased N_2O in *F. moluccana* forests ($P = 0.003$) but not *M. polymorpha* forests ($P = 0.35$).

The ratio of N_2O to NO, thought to be positively related to microbial rates of denitrification (Davidson et al. 2000), varied most with the presence or absence of *E. coqui* and was highest (N_2O:NO = 3) in *F. moluccana* stands that had

Table 2. Soil properties in *M. polymorpha* and *F. moluccana* stands, with and without the presence of *E. coqui*. Values are mean (standard deviation).

Soil variable	No *E. coqui*		*E. coqui*	
	M. polymorpha	*F. moluccana*	*M. polymorpha*	*F. moluccana*
Gravimetric soil moisture (g/g)	2.2 (1.6)	1.2 (0.5)	2.2 (0.4)	1.7 (0.1)
pH	5.6 (0.3)	5.7 (0.8)	5.5 (0.3)	5.5 (0.3)
Soil NH_4^+ (μg N/g)	24.2 (16.6)	25.8 (15.9)	22.6 (2.6)	20.1 (0.2)
Soil NO_3^- (μg N/g)	9.9 (14.1)	60.5 (67.0)	6.1 (8.1)	33.9 (12.7)
Net N mineralization (μg $N \cdot g^{-1} \cdot d^{-1}$)	1.9 (3.1)	4.6 (0.1)	4.7 (1.8)	6.6 (2.4)
Net nitrification (μg $N \cdot g^{-1} \cdot d^{-1}$)	2.6 (3.7)	5.6 (1.0)	3.2 (4.3)	6.3 (1.0)
Nitrification potential (μg $N \cdot g^{-1} \cdot h^{-1}$)	0.9 (1.2)	1.2 (0.4)	0.4 (0.4)	1.7 (0.1)

Notes: Mean and standard deviation are calculated for two sites within each *E. coqui* × tree species category. Values for each site are averages of three soil samples, each composed of three soil cores.

been invaded by *E. coqui* and lowest (N_2O:NO < 1) in *F. moluccana* stands that were free of *E. coqui*. However, *E. coqui* was not significantly related to soil properties in either forest type, as shown through factor analysis (Fig. 3b). *E. coqui* was unrelated to variance along PC 1 (MANOVA, $P = 0.56$) or PC 2 (Mann–Whitney *U* test, $P = 0.84$). Similarly, *E. coqui* had no significant effect on soil NO emissions or the ratio of soil N_2O:NO (Fig. 2b, c).

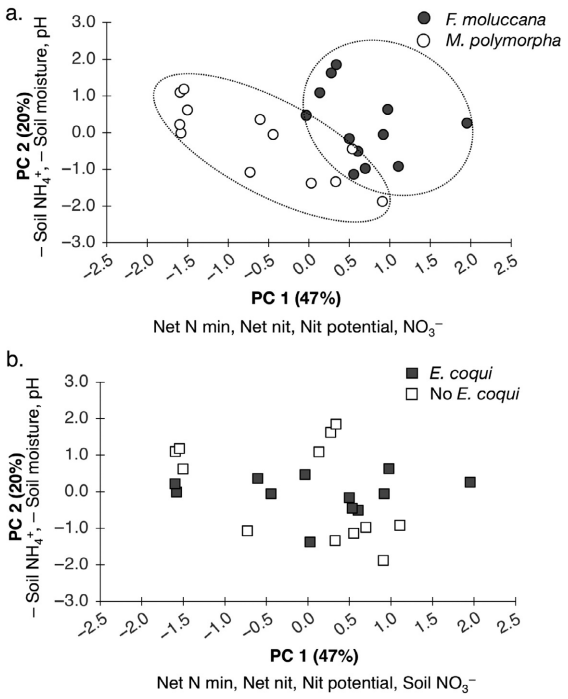

Fig. 3. Results from principal components analyses of soil variables from (a) *M. polymorpha* or *F. moluccana* stands and (b) forest stands that were either invaded or not invaded by *E. coqui*. Shown on each axis is the percent of explained variance and the soil variables that compose each axis (see factor loadings in Table 3).

Across native and invaded forests, site-averaged N_2O fluxes were strongly and significantly related to net potential N mineralization (2 of 3 models; Table 4) but unrelated to other soil properties or processes. NO fluxes were positively and significantly related to NH_4^+ concentration and nitrifier population size (estimated from nitrification potential), and they were negatively related to soil moisture (adjusted $R^2 = 0.96$, $P < 0.001$). Total soil N-oxide fluxes were significantly and positively related to soil NO_3^- and nitrifier population size. As expected, the ratio of N_2O to NO was positively and significantly related to soil moisture (1 of 3 models), but it was also negatively related to NH_4^+ concentration (2 of 3 models) and soil NO_3^- (1 of 3 models).

Discussion

This study is the first to quantify the influence of a non-native animal invasion on soil N-oxide gas emissions in the field, and one of only a few to characterize soil N_2O and NO emissions from an introduced non-native plant species. Both species studied here belong to functional groups of organisms that would theoretically be expected to affect ecosystem functioning through their ability to increase the supply of limiting resources (*F. moluccana*, an N_2 fixer), and alter trophic structure (*E. coqui*, a predator). Additionally, the unique characteristics of the Hawaiian Islands make them a model system for ecological research because the native biota is species-poor compared to continental habitats, species

Table 3. Principal components analysis of soil properties within *M. polymorpha* and *F. moluccana* stands, with and without the presence of *E. coqui*.

Variable	Factor loadings		Transformation used
	PC1	PC2	
Net potential nitrification (μg $N \cdot g^{-1} \cdot d^{-1}$)	**0.887**	−0.09	x
Net potential N mineralization (μg $N \cdot g^{-1} \cdot d^{-1}$)	**0.793**	0.09	x
Nitrification potential (μg $N \cdot g^{-1} \cdot h^{-1}$)	**0.693**	0.062	x
Soil NO_3^- (μg N/g)	**0.645**	−0.28	√(x)
Soil NH_4^+ (μg N/g)	0.097	**−0.841**	x
Gravimetric soil moisture (g/g)	−0.174	**−0.864**	x
pH	−0.207	**0.766**	x
Eigenvalue	3.3	1.4	
Fraction of variance explained (%)	47	20	

Note: Bold values in Table 3 are factor loadings > 0.6 or < −0.6.

invasions are relatively well monitored (BIISC 2018, HEAR 2018), and because it is possible to find sites where common confounding factors can be held relatively constant (e.g., climate, topography, the native species pool, geology/soils, time since disturbance; Vitousek 2004). Using this natural laboratory, our research shows that established non-native species that substantially increase the rate of ecosystem N cycling can have effects on atmospheric properties by increasing the emissions of two important trace gases, N_2O and NO, from soil to the atmosphere.

As expected, higher rates of soil N-oxide emissions under *F. moluccana* compared to *M. polymorpha* were strongly related to net rates of microbial N mineralization and nitrification, both of which are likely to be stimulated by large inputs of N-rich, highly decomposable litter and altered invertebrate communities in *F. moluccana* stands (Hughes and Uowolo 2006). However, contrary to expectations, invasion by *E. coqui* increased N_2O emissions in stands dominated by N-rich *F. moluccana*, but not in N-poor stands of *M. polymorpha*.

In previous work at our study sites, decomposition of a common litter was increased by two-fold in *F. moluccana* compared to native forests, and rates were further accelerated, although only

Table 4. Results of regression analyses between site-averaged soil N-oxide gases, soil properties, and soil nutrient transformations in native (*M. polymorpha*) and invaded (*E. coqui* and/or *F. moluccana*) Hawaiian rainforests.

Dependent variable	Transformation used	Model number	Akaike weight (%)	Soil predictor variable	β	Sig.†	Adj. R^2	*P*†
N_2O	√(x + 0.5)	1	31	Net N mineralization	0.71	0.05	0.42	0.05
		2	19	Net N mineralization	0.83	0.02	0.61	0.04
				Gravimetric soil moisture	−0.49	0.11		
		3	16	Nitrification potential	0.64	0.09	0.32	0.09
NO	Ln(x + 0.5)	1	70	Gravimetric soil moisture	−0.88	0.001	0.96	0.001
				NH_4^+	0.99	0.001		
				Nitrification potential	0.54	<0.01		
N_2O + NO	Ln(x + 0.5)	1	60	NO_3^-	0.71	<0.01	0.88	<0.01
				Nitrification potential	0.49	0.02		
		2	20	NO_3^-	0.88	<0.01	0.94	<0.01
				Nitrification potential	0.48	<0.01		
				pH	0.28	0.07		
N_2O/NO	Ln(x)	1	37	Gravimetric soil moisture	0.69	0.05	0.72	0.02
				NH_4^+	−1.17	0.01		
		2	21	NO_3^-	−0.73	0.04	0.46	0.04
		3	18	NH_4^+	−0.72	0.04	0.44	0.04

Note: Shown are all acceptable models based on an information criterion approach using Akaike weights.
† *P* is *P* value of the full model; Sig. is *P* value of each variable in the model.

moderately, by ~30% with the presence of *E. coqui* (Norman 2008). Supporting similar trends, Tuttle et al. (2009) showed that invertebrate communities were more affected by nutrient-rich *F. moluccana* litter (twofold to fourfold increases in non-native fragmenters and ants) than the presence of *E. coqui* (30–40% lower abundance of ants and microarthropods). Our findings suggest that, while an invasive predator can speed ecosystem nutrient cycling in both native *M. polymorpha* and invaded *F. moluccana* forests, fluxes of N_2O are only likely to increase when rates of N cycling are substantially elevated, as found in *F. moluccana* stands.

Our results suggest that, following invasion of an introduced species, a measurable change in soil N_2O flux requires N enrichment beyond a particular threshold. This hypothesis is supported by a prior study in native and N-fixer invaded forests of Hawai'i, where soil N_2O emissions were elevated only when stand-level foliar C:N declined below 16:1 (Hall and Asner 2007). Together, our results and others show that an invasive, non-native amphibian increases ecosystem nutrient cycling and N_2O emissions in N-enriched forests, but its effects are secondary to the dramatic change in resource provisioning by an invasive, non-native N_2-fixing plant. Although related studies show that these non-native species alter nutrient cycling by different mechanisms (i.e., *F. moluccana* increases ecosystem N supply, and *E. coqui* increases the speed of labile N release from detritivores), this research suggests that species that substantially enrich ecosystem N availability for nitrifying and denitrifying microorganisms will in turn increase emissions of soil N-oxides.

Although variance within and between sites was high, average N_2O:NO ratios were ~1 (range 0.2–3.1) in forests that were invaded by *E. coqui* and ~0.4 (range 0.04–1.6) in forests that were not. Drawing from the conceptual hole-in-the-pipe (HIP) model, the ratio of N_2O:NO has been used to estimate the relative source of these two gases, with ratios >1 occurring in a more reductive soil environment that supports denitrification, and N_2O:NO < 1 occurring in a more oxidative environment that supports nitrification (Davidson et al. 2000). Much has been learned over the last 30 yr of testing the HIP model, especially about the complexity of microbial community and abiotic processes related to soil N_2O and NO production (van Groenigen et al. 2015). However, the framework of the HIP model continues to be supported across tropical and subtropical forest soils worldwide, at least those in which bacterial denitrification and ammonia oxidation are dominant (van Lent et al. 2015, Diem et al. 2017).

Although we did not measure denitrification in this study, N_2O:NO ratios in forests invaded by *E. coqui* are consistent with increases in this heterotrophic process, which could be stimulated by coqui-driven acceleration of dissolved organic C (DOC) and DON inputs (Beard et al. 2002). Although invasion of *E. coqui* is also associated with other trophic changes, such as increased abundance of non-native birds who likely eat *E. coqui* and potentially other small vertebrates as prey (Smith et al. 2018), the consequences of these additional food web alterations are currently unknown. We found that the ratio of N_2O to NO emission was positively related to soil moisture (Table 4). Based on these results, presence of *E. coqui* may also alter soil structure in ways that promote anaerobic microsites for denitrification, by changing the composition and abundance of detritivorous invertebrate communities, even moderately (Tuttle et al. 2009).

This study documents substantial increases in N-oxide emissions from *F. moluccana*-invaded areas and, to a lesser extent from *E. coqui* invaded areas on N-poor substrates in Hawai'i, but the extent to which these invasions are altering N-oxide emissions annually, or across larger scales, is unclear. Evidence from extensive invasion of another N-fixing tree in Hawai'i, *Morella faya*, suggests there may be effects of *F. moluccana* populations on N-oxide emissions at regional scales. In a cooler montane Hawaiian wet forest, Hall and Asner (2007) found that the non-native N-fixing tree, *M. faya*, increases regional N-oxide emissions ~16 times the rate compared to native forests. Leaf N content doubled following *M. faya* invasion and was strongly correlated to both soil N_2O and NO emissions. *F. moluccana* has a higher leaf N content than *M. faya* but a lower leaf area index, resulting in similar canopy N pools (2.8 g N/m^2 *F. moluccana* vs. 3.0 g N/m^2 *M. faya*; Asner et al. 2008*b*).

The effects of plants on air quality are well known through their production of volatile organic compounds that contribute to tropospheric ozone formation (Fiore et al. 2005).

However, the consequences of biogenic gas emissions from soils on regional air quality are less well understood. Soil N_2O fluxes were similar between *F. moluccana* and those reported for *M. faya* but soil NO fluxes from *F. moluccana* were an order of magnitude higher than *M. faya* (1–30 ng NO-N·cm^{-2}·h^{-1} *F. moluccana* vs. 0.2–1 ng NO-N·cm^{-2}·h^{-1} *M. faya*; Hall and Asner 2007). Because NO emissions are strongly influenced by temperature when N is in ample supply for microorganisms (Hall et al. 1996), it is likely that the warmer soils of lowland *F. moluccana* stands contributed to elevated rates of nitrification and NO fluxes compared to N_2-fixing tree invasions in cooler, montane areas of Hawai'i. Alternatively, higher total N-oxide emissions may be related to likely higher rates of litterfall from lowland *F. moluccana* compared to montane *M. faya* (Hughes and Denslow 2005, Hughes et al. 2014). In one example, extensive invasion across the southern United States by the N_2-fixing vine kudzu (*Pueraria montana*) increased the number of regional high ozone days relative to native vegetation in part by doubling soil NO fluxes, which were in the same range of emissions from *F. moluccana* (Hickman et al. 2010).

Falcataria moluccana and *E. coqui* are currently widespread in mesic to wet forests across all the major Hawaiian Islands (Watson 2017, USGS 2018). However, our study was conducted during one field season (summer 2006), in a limited set of sites on young lava substrates in eastern Hawai'i. Thus, it is unclear how well our findings extrapolate to other sites in the Hawaiian Islands or other tropical ecosystems. *E. coqui* increases rates of nutrient cycling even in fertile subtropical forests in its native Puerto Rico (Beard et al. 2002), but less is known about the potential impacts of *F. moluccana* invasion in more diverse tropical plant communities across the Pacific Islands (Kueffer et al. 2007). For example, variation in soil fertility and N-oxide emissions among our sites was likely related to differences in substrate age and type, which are known to influence substrate weathering and ecosystem processes (Aplet et al. 1998). N-fixers significantly increase rates of nutrient cycling in ecosystems where N limits primary production, but they may not significantly alter N cycling and N-oxide fluxes in other N-rich tropical systems. In support of this idea, Hughes et al. (2014) suggest that differences between native and N_2-fixer invaded forests decline with substrate (or soil) age, as was shown for aboveground C density between *M. polymorpha*- and *F. moluccana*-dominated sites (Hughes et al. 2014).

Across longer time scales of ecosystem development, atmospheric N inputs and rock weathering decrease available pools of P relative to N in many tropical ecosystems, leading to N and P co-limitation of plant growth in moderately weathered soils, and P limitation in forests on highly weathered substrates (Vitousek et al. 1997). P limitation could limit biological N_2 fixation and thus limit N availability and N-oxide emissions from soil beneath N_2-fixers (Vitousek and Howarth 1991). However, recent evidence suggests that N_2 fixers can invest in N-rich enzymes to liberate soil P resources and thus may be insensitive to P limitation (Wang et al. 2007, Baribault et al. 2012). Furthermore, Hall and Matson (2003) showed that rates of soil nitrification, populations of nitrifying microorganisms, and N-oxide emissions were accelerated more by experimental N fertilization on older, P-poor substrates (N-rich soils) compared to younger substrates (N-poor soils). If *F. moluccana* and *E. coqui* invasions elsewhere speed N cycling relative to the forests they invade, we would expect the consequences for soil N_2O and NO emissions to be significant, especially in sites where N-limitation is alleviated and soil microbial nitrifying populations are well established.

Acknowledgments

We are thankful to an internal USDA peer review and two anonymous reviewers whose comments substantially improved this manuscript. All authors conducted the fieldwork for this research and contributed to the manuscript. Sharon J. Hall and R. Flint Hughes conceived of the research question, R. Flint Hughes identified the field sites, and David P. Huber conducted laboratory and data analyses. We are grateful to Research Associate Laura Steger for statistics, data management, and GIS assistance and ASU undergraduate Samantha Straus for field sampling assistance in Hawai'i. We also thank Pamela Matson and Peter Vitousek for use of the field laboratory at Hawai'i Volcanoes National Park, use of the chemiluminescent instrument, and for inspirational conversations related to this work. This project was supported by a grant from the Andrew W. Mellon Foundation to Sharon J. Hall. Any use of trade, product, or firm names is for descriptive purposes only and does not imply

endorsement by the U.S. Government. USDA is an equal-opportunity provider and employer.

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