

A non-native invasive grass increases soil carbon flux in a Hawaiian tropical dry forest

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Abstract

Non-native plants are invading terrestrial ecosystems across the globe, yet little is known about how invasions impact carbon (C) cycling or how these impacts will be influenced by climate change. We quantified the effect of a non-native C₄ grass invasion on soil C pools and fluxes in a Hawaiian tropical dry forest over 2 years in which annual precipitation was average (Year 1) and ~60% higher than average (Year 2). Work was conducted in a series of forested plots where the grass understory was completely removed (removal plots) or left intact (grass plots) for 3 years before experiment initiation. We hypothesized that grass invasion would: (i) not change total soil C pools, (ii) increase the flux of C into and out of soils, and (iii) increase the sensitivity of soil C flux to variability in precipitation. In grass plots, grasses accounted for 25–34% of litter layer C and ~70% of fine root C. However, no differences were observed between treatments in the size of any soil C pools. Moreover, grass-derived C constituted a negligible fraction of the large mineral soil C pool (<3%) despite being present in the system for ≥50 years. Tree litterfall was ~45% lower in grass plots, but grass-derived litterfall more than compensated for this reduction in both years. Annual cumulative soil-surface CO₂ efflux (R_{soil}) was ~40% higher in grass plots in both years, and increased in both treatments by ~36% in the wetter Year 2. Despite minimal grass-derived mineral soil C, >75% of R_{soil} in grass plots was of C₄ (i.e. grass) origin. These results demonstrate that grass invasion in forest ecosystems can increase the flux of C into and out of soils without changing total C pools, at least over the short term and as long as the native tree canopy remains intact, and that invasion-mediated changes in belowground C cycling are sensitive to precipitation.

Keywords: belowground carbon cycling, Hawaii, invasive species, litterfall, litter decomposition, *Pennisetum setaceum* ('fountain grass'), soil carbon pools and fluxes, soil-surface CO₂ efflux ('soil respiration')

Received 11 August 2006; revised version received 4 October 2007 and accepted 16 October 2007

Introduction

Non-native plants are invading nearly all terrestrial ecosystems and are among the most pervasive elements of global change biology (Vitousek *et al.*, 1997; Mack *et al.*, 2000; Didham *et al.*, 2005). Previous work has documented that invasions can have substantial impacts on both the structure and function of native

ecosystems (see Ehrenfeld, 2003). Most work to date has concentrated on nutrient dynamics, but plant invasions also have the potential to alter the terrestrial carbon (C) cycle at local to regional scales (Litton *et al.*, 2006). However, few studies have examined how invasive species impact C transfers across the soil–plant–atmosphere interface. Moreover, interactions between invasive species and climate have yet to be explored within the context of C cycling.

Understanding the impacts of plant invasions on belowground C cycling is critical for predicting how invasive species will affect the exchange of C between the atmosphere and terrestrial ecosystems. Soil-surface CO₂ efflux (R_{soil} , 'soil respiration') is the largest flux of

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C in forests outside of gross primary productivity (Law *et al.*, 1999; Janssens *et al.*, 2001) and is the dominant component of ecosystem respiration (Bolstad *et al.*, 2004; Curtis *et al.*, 2005; Yuste *et al.*, 2005). In turn, ecosystem respiration can determine the overall C balance of terrestrial ecosystems (Valentini *et al.*, 2000). Globally, annual R_{soil} is $\sim 10 \times$ greater than emissions of atmospheric CO_2 from fossil fuel combustion and deforestation combined (Schlesinger & Andrews, 2000). Because soils are the single largest pool of C in terrestrial ecosystems (Jobbágy & Jackson, 2000; Schlesinger *et al.*, 2000), even small changes in the flux of C from soils to the atmosphere as a result of invasion could have large impacts on atmospheric composition and global climate.

The addition of a new species via invasion has the potential to fundamentally alter ecosystem processes. Changes in species abundance and diversity can strongly influence ecosystem function because individual species differ in the rate at which resources are acquired, the efficiency with which those resources are utilized to produce biomass, and interactions with the physical environment and other organisms (Chapin *et al.*, 1997). Non-native invasive species most likely to alter ecosystem processes are those that: (i) differ from natives in resource acquisition and utilization, (ii) alter trophic structures, or (iii) influence disturbance regimes (Vitousek, 1990). The success of invasive plants has been at least partially linked to all three of these mechanisms (Mack *et al.*, 2000; Daehler, 2003; Callaway & Ridenour, 2004).

Island ecosystems are particularly susceptible to plant invasions (Gimeno *et al.*, 2006). In Hawaii, it is estimated that $< 10\%$ of the original tropical dry forest remains (Bruegmann, 1996), primarily as a result of the introduction of invasive species and changes in disturbance regimes (Hughes *et al.*, 1991; D'Antonio & Vitousek, 1992). Native grasses were presumably a minor component of this system, but today existing fragments of dry forest on the Island of Hawaii are often heavily invaded by the non-native C_4 perennial bunchgrass *Pennisetum setaceum* (Forssk.) Chiov. ('fountain grass'). Previous work in these forest fragments has documented that grass invasion into otherwise intact native dry forest increases understory biomass $\sim 7 \times$ but has no impact on total aboveground C pools because of the large amount of woody biomass in trees (Litton *et al.*, 2006). However, there is often poor correlation between biomass and C flux in forests (Litton *et al.*, 2007), and information on both C pools and fluxes is needed to develop a comprehensive understanding of how invasive species impact C cycling.

Here, we use *P. setaceum* invasion into Hawaiian tropical dry forest to address how a non-native invasive

plant impacts belowground C cycling by examining soil C pools and fluxes in the presence and absence of grass invasion. Specifically, our objectives were to compare plots invaded by *P. setaceum* and plots where the grass had been removed for 3 years before experiment initiation to quantify: (i) C pools in the litter layer, roots, microbial biomass and mineral soil, (ii) C fluxes in R_{soil} , aboveground litterfall and litter decomposition, (iii) the relationship between R_{soil} and leaf area index (LAI), and (iv) the C_3 vs. C_4 contributions to soil C pools and fluxes. In addition, our measurements span a 2-year time period in which annual rainfall differed by $\sim 60\%$, allowing us to explore how variation in precipitation interacts with plant invasion to impact belowground C cycling. We hypothesized that: (H1) grass invasion would result in minimal changes in the size of soil C pools (Ehrenfeld, 2003), as loss of forest C_3 -C would be replaced by new grass C_4 -C (Bashkin & Binkley, 1998; Binkley & Resh, 1999; Osher *et al.*, 2003); (H2) the flux of C into and out of soils would increase with invasion, and the grass contribution to these fluxes would be proportional to its contribution to total ecosystem C pools; and (H3) increased precipitation would increase all components of soil C flux, both with and without grass, but the relative increase in flux would be higher in grass-invaded forests (i.e. increased sensitivity to changes in precipitation with grass invasion) because grass production would be more limited by near-surface soil water availability.

Materials and methods

Study area

The study was carried out in the Kaupulehu Dry Forest Preserve (608 m elevation, $19^\circ 46' 05'' \text{N}$, $155^\circ 56' 19'' \text{W}$), located on the northwest slope of Hualalai Volcano in the district of North Kona, Island of Hawaii (see Litton *et al.*, 2006). Based on aerial photography, *P. setaceum* has been a dominant component of this landscape since at least the early 1960s, and likely decades earlier. Experimental plots were located in a 7–8 m tall mature forest that is dominated by two native evergreen trees, *Diospyros sandwicensis* (A. DC) Fosb. and *Psydrax odorata* (Forst. F.) A. C. Sm. and S. Darwin, which together account for $> 91\%$ of the total basal area and stem density (Litton *et al.*, 2006).

Parent material of the study site consists of tholeiitic basalt laid down primarily as 'a'a lava flows (rough and blocky) ~ 1500 – 3000 years ago (Moore *et al.*, 1987). Soils are shallow, highly organic (fine fraction of mineral soil $> 30\%$ C), $> 50\%$ coarse rocks (> 5 mm) by volume, with an average pH of 7.6 (± 0.1), and are classified as euic, isothermic, shallow, Lithic Ustifolist

(USDA-NRCS classification). The high soil C content and vegetation characteristics suggest that our study area occurs on the far upper end of the estimated substrate age range (see Stemmermann & Ihsle, 1993), and may well be older.

Long-term mean annual rainfall for the study area is ~700–750 mm, with great year-to-year variability (Giambelluca *et al.*, 1986; Litton *et al.*, 2006). Mean annual rainfall from 1999 to 2005 was 514 mm, with a low of 225 mm in 1999 and a high of 1249 mm in 2004. Large rainfall events can occur during any month, but summers tend to be drier than winters. Total annual precipitation was 732 mm during Year 1 of this study (May 1, 2003 to April 30, 2004) and 1189 mm during Year 2 (May 1, 2004 to April 30, 2005). At a nearby weather station (~10 km; 709 m elevation; 19°47'42"N, 155°50'43"W), mean daily temperature for 2004–2005 was 20.0 °C, with a mean daily maximum of 25.6 °C and a mean daily minimum of 15.1 °C.

Experimental design

Work was conducted in a series of eight forested plots (12 m × 12 m) that were randomly established in July 2000 – the grass understory was removed from half of the plots (removal plots) and left intact in the remaining four plots (grass plots). Tree basal area and stem density did not differ between treatments ($P > 0.78$; Table 1). In June 2003, an additional 4 m buffer was established around plots to minimize edge effects (20 m × 20 m plots with all measurements taken in the central 12 m × 12 m). Grass was removed from removal plots and buffer areas with a general herbicide and manual extraction of aboveground biomass (Cordell *et al.*, 2002). Grass seedlings were pulled from removal plots on a regular basis. At the start of the study, 3 years after plot

establishment, the understory in grass plots was a monospecific cover of *P. setaceum*, while the understory in removal plots consisted of scattered native shrub, vine and graminoid species which established following grass removal [*Lipochaeta subcordata* A. Gray; *Cocculus trilobus* (Thunb.) DC; *Sicyos lasiocephalus* Skottsb.; and *Mariscus hillebrandii* (Boeck.) T. Koyama].

Soil C pools

C pools were quantified in litter, roots, mineral soil, and microbial biomass in three plots per treatment in July 2003 (Year 1), and again in March 2005 (Year 2; minus roots). Litter layer C (i.e. forest floor) was estimated by collecting all surface litter down to the mineral soil interface within three randomly placed 0.0625 m² frames plot⁻¹. Litter samples were dried to a constant weight in a forced air oven, ground in a Wiley Mill to pass a 0.5 mm² mesh (size 40), and analyzed for C content on a Fisons NA-1500 elemental analyzer (Fison Instruments, Milan, Italy).

For root (fine and coarse) and mineral soil C, two randomly located soil pits in three plots of each treatment were excavated under the 0.0625 m² litter layer quadrats by digging down to consolidated, dense basalt at depths ranging from 15 to 30 cm. The content of each pit passing through a 2 mm sieve was classified as the mineral soil fine fraction (<2 mm). All remaining material was separated into fine (<2 mm) and coarse (≥2 mm) roots, mineral soil coarse fraction (≥2 and <5 mm), and rocks (≥5 mm). Fine roots correspond to total fine root biomass (live + dead). Fine and coarse roots were dried, ground and analyzed for C content as above. Our estimates of root C are underestimates because they do not account for C in tree root crowns or deep roots in cracks extending below the consolidated basalt. Well-mixed subsamples of the mineral soil fine fraction were dried in a forced air oven to a constant weight, ground for 24 h on a roller mill, and analyzed for C content as above. Organic matter in the mineral soil coarse fraction and rocks (fine roots and soil organic matter in the porous rocks) was estimated by processing subsamples in a muffle furnace at 400 °C for 12 h (Nelson & Sommers, 1996); percent C of combusted material was estimated as 33.6% (mean of all mineral soil fine fraction samples). Soil bulk density of each fraction, needed to scale concentrations to mass per unit area, was determined by backfilling each pit with fine cinder to determine total pit volume and quantifying the volume of the pit occupied by each fraction.

Microbial biomass C (C_{mic}) was quantified on well-mixed samples of the mineral soil fine fraction at both sampling dates using the chloroform fumigation

Table 1 Stand characteristics of the tropical dry forest study site on the Island of Hawaii with (grass) and without (removal) *Pennisetum setaceum*, a non-native invasive grass

	Removal	Grass
Basal area (cm ² m ⁻²)	20.8 (1.4) ^a	22.0 (3.5) ^a
Stem density (trees ha ⁻¹)	3420 (915) ^a	3368 (569) ^a
LAI (m ² m ⁻²) – Year 1		
Overstory (>1.33 m)	1.89 (0.16) ^a	1.37 (0.12) ^b
Understory (<1.33 m)	0.21 (0.10) ^a	1.67 (0.18) ^b
LAI (m ² m ⁻²) – Year 2		
Overstory (>1.33 m)	1.86 (0.17) ^a	1.30 (0.10) ^b
Understory (<1.33 m)	0.21 (0.10) ^a	2.38 (0.35) ^b

All values are treatment means (±1 SE; $n = 4$); means with the same letter within a row do not differ at $\alpha = 0.05$.

LAI, leaf area index.

extraction method modified from Vance *et al.* (1987). We used an identical procedure to that described in Litton *et al.* (2003), except that air-dried samples were rewetted to 50% water-holding capacity and incubated for 7–10 days before extraction, all extracts were diluted 5 × before C content analysis, and a k_{EC} of 0.45 was used due to high organic matter content in our soils (Sparling *et al.*, 1990).

Soil-surface CO₂ efflux

Soil-surface CO₂ efflux (R_{soil}) was measured monthly at eight points in each plot with a LI-COR 6400 portable photosynthesis system and LI-COR 6400-09 soil respiration chamber (LI-COR, Lincoln, NE, USA). We used permanently placed 10 cm diameter polyvinyl chloride (PVC) collars inserted 4 cm into the litter and mineral soil. Two measurements were made at each point by drawing down chamber CO₂ concentration below ambient concentrations at the soil surface, and measuring chamber CO₂ concentration from 10 ppm below ambient to 10 ppm above ambient.

We quantified the temperature sensitivity and diel course of R_{soil} by measuring efflux on all eight collars in one plot of each treatment every 3 h for 24 h in July 2004. In addition, we quantified the effects of discrete pulse precipitation events on R_{soil} by measuring efflux 2 days before a large pulse event (95 mm), 1 day after the pulse event, and every 2–3 days thereafter for a month on all eight collars in one plot of each treatment.

To explore the relationship between R_{soil} and environmental variables, we quantified soil water potential (Ψ_{soil}) and soil temperature throughout the experiment in three plots of each treatment. Ψ_{soil} at 10 cm was measured weekly at four locations per plot using individually calibrated *in situ* thermocouple psychrometers (PST-55) and a microvolt meter (HR-33T) operating in dew point depression mode (Wescor, Logan, UT, USA). Continuous, hourly measurements of soil temperature at 10 cm were made on three locations per plot with high-resolution temperature sensors (TMC6-HD; Onset Computer Corporation, Bourne, MA, USA).

Annual cumulative R_{soil} ($\text{g C m}^{-2} \text{yr}^{-1}$) was estimated using two approaches that rely on linear interpolation to fill in missing daily values. In the ‘monthly interpolation’ approach we used linear interpolation between monthly R_{soil} measurements, while in the ‘weekly interpolation’ approach we supplemented monthly R_{soil} measurements with weekly R_{soil} estimates based on the relationship between Ψ_{soil} and R_{soil} (see ‘Results’). R_{soil} exhibited no temperature or diel response (see ‘Results’), so measured instantaneous rates

($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) were scaled to daily C flux rates ($\text{g C m}^{-2} \text{day}^{-1}$) with no temperature or diel correction.

Aboveground litterfall and litter decomposition

Tree aboveground litterfall was collected monthly in all plots from eight 0.1764 m² traps. Litter from each trap was dried at 70 °C to a constant mass in a forced air oven and weighed. The C content of litterfall was analyzed as above on a subset of composite samples, and the mean value (43.7%) was used to quantify aboveground litterfall C flux. For grass and all other understory vegetation, annual litterfall was assumed equivalent to annual aboveground net primary production, which was estimated by summing positive increments in live and dead standing biomass (trough-peak analysis) in grass plots (grasses), and with clip plots during peak standing biomass in removal plots (all other understory vegetation) (Scurlock *et al.*, 2002). Grass biomass was 44.5% C.

Litter decomposition of *D. sandwicensis* and *P. setaceum* foliage was assessed *in situ* for 10 months with 100 cm² litterbags made of 2 mm fiberglass mesh. Recently senesced foliage was collected in all plots, air dried and composited by species and treatment. A well-mixed subsample of each species and treatment combination was dried to a constant mass in a forced air oven to determine percent moisture for correcting initial litter mass. Approximately 1.5 g of intact, air-dried litter was weighed and placed in each litterbag, and four litterbags of each species and treatment combination were placed in plots in March, 2004 (*D. sandwicensis* in removal and grass plots, and *P. setaceum* in grass plots). Initial litter chemistry (% C, N, lignin, cellulose and hemicellulose) was determined on a well-ground subsample of each litter type and treatment combination using sequential C fraction analysis (Ryan *et al.*, 1990). Litterbags were collected after 10 months, soil was carefully removed from each sample, samples were dried to a constant mass, weighed, and species replicates within a given plot were composited, ground in a Wiley mill, and analyzed for percent ash in a muffle furnace and CHN content on a Costech ECS 4010 Elemental Combustion Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA).

C₄ contribution to soil C pools and fluxes

The grass contribution (C₄ grass) to soil C pools in the research area (C₃ forest) was assessed using a standard two-member isotope mixing model of the form

$$f_A = \frac{\bar{\delta}_M - \bar{\delta}_B}{\bar{\delta}_A - \bar{\delta}_B}, \quad (1)$$

where $\bar{\delta}_M$, $\bar{\delta}_A$, and $\bar{\delta}_B$ are the mean $\delta^{13}\text{C}$ isotopic signatures of the mixture (M) and sources A and B, respectively, and f_A is the proportion of A in M (Phillips & Gregg, 2001). The model was run separately for the litter layer, roots and mineral soil fine fraction. End members (A and B) for each mixing model came from measured $\delta^{13}\text{C}$ values of: (i) litter layer C – *P. setaceum* and tree litter; (ii) root C – *P. setaceum* and tree roots; and (iii) mineral soil C – *P. setaceum* roots and preinvasion soil organic matter (no C₄ contribution) calculated as the $\delta^{13}\text{C}$ signature of tree foliage enriched by 2‰. The 2‰ enrichment assumption for mineral soil end member designation is based on: (i) a nearby (<5 km), uninvaded dry forest with similar vegetation (see Litton *et al.*, 2006), where the isotopic signature of bulk mineral soil is 2.04‰ enriched compared with *in situ* tree foliage (C. M. Litton, unpublished data); and (ii) the commonly observed phenomenon in forest ecosystems that mineral soil is enriched 1–3‰ compared with foliage (Ehleringer *et al.*, 2000).

We also quantified the grass (C₄) contribution to R_{soil} using a Keeling plot approach (Pataki *et al.*, 2003) on two locations in one plot of each treatment in July of 2004. A custom soil chamber (0.02 m³) was placed over the soil surface and sealed with an O-ring made from nylon hose filled with fine sand. Soil chamber CO₂ concentrations were determined by circulating air through the chamber with a LI-COR 6400 portable photosynthesis system. Small samples of soil chamber air were extracted periodically via an inline septum using a 10 mL syringe over 5–10 min as chamber CO₂ concentration increased from ambient to ~550 ppm. Samples were stored in 10 mL Exetainer vials (Labco, UK) that had been flushed and filled with He. All samples were analyzed for $\delta^{13}\text{C}$ within 60 h of field sampling on a Finnigan MAT Delta-S isotope ratio mass spectrometer with precon at the Stable Isotope Ratio Facility for Environmental Research (University of Utah). The y -intercept of the resulting least squares linear regression between $\delta^{13}\text{C}$ (y -axis) and the inverse of CO₂ concentration ($1/[\text{CO}_2]$; x -axis), the Keeling plot (Keeling, 1958), predicts the $\delta^{13}\text{C}$ value of CO₂ entering the chamber from the soil. A two-member mixing model was used to determine the C₄ and C₃ contributions to R_{soil} (Phillips & Gregg, 2001). End members in the mixing model were measured $\delta^{13}\text{C}$ values: grass roots for the C₄ component and grass-free forest mineral soil and tree roots for the C₃ component. For the latter end member, we assumed that 50% of R_{soil} was autotrophic (root, root exudates, mycorrhizae) and 50% heterotrophic (mineral soil) origin (see Bond-Lamberty *et al.*, 2004), such that the C₃ end member was calculated as the average of root and mineral soil $\delta^{13}\text{C}$.

Contribution to aboveground litterfall by trees (C₃) and grasses (C₄) was quantified by simply dividing litterfall flux of grasses or trees by total aboveground litterfall (tree + grass).

Leaf area index

LAI (m² m⁻²) was estimated in August of both years for the overstory and understory between 4:00 and 6:00 hours on days with widespread cloud cover. Overstory LAI was estimated with a pair of LAI-2000 plant canopy analyzers (Li-Cor Inc., Lincoln, NE, USA), where diffuse solar radiation was measured below and above the canopy simultaneously. Below canopy measurements were taken directly above the understory vegetation at 1.33 m on eight points per plot on a systematic grid. For understory LAI, we used the LAI-2000 operating in 1 unit mode, by taking measurements at the soil surface and at 1.33 m. We corrected understory LAI estimates to include only live foliage (LAI multiplied by %biomass in live tissues) by harvesting standing grass biomass from fifteen 0.25 m² plots and quantifying the live:dead biomass ratio, with the assumption that live and dead foliage had similar mass per area, as well as reflective and transmittance properties. Field data were analyzed using FV2000 software masking the outermost ring (Li-Cor Inc.). No corrections were made to account for woody biomass in the canopy and, therefore, our overstory LAI values refer to plant area, and likely overestimate leaf area (Kalácska *et al.*, 2005).

Statistical analysis

The sample unit for this study was the plot and all data presented are mean plot values ($n = 3$ or $n = 4$ depending on the measurement). All statistical analyses were performed in SPSS 10.0 for WINDOWS (SPSS Inc., Chicago, IL, USA) at an $\alpha = 0.05$. All data were tested for assumptions of normality and equality of variance. Tests for differences among treatments in stand structural characteristics, soil C pools, and the C₄ contribution to soil C pools and fluxes were performed using one-way analysis of variance (ANOVA) – except for the C₄ contribution to R_{soil} , where treatment differences were analyzed with an independent sample t -test. Treatment and time effects for R_{soil} (instantaneous, daily and yearly), F_A (monthly and yearly), soil temperature, and Ψ_{soil} were analyzed with a repeated measures general linear model. Linear and nonlinear regression techniques were used to explore the relationships between R_{soil} and soil temperature, soil water potential, and LAI.

Table 2 Soil carbon pools (g C m^{-2} to 30 cm depth) in grass and grass removal plots

Component	Soil C (g C m^{-2})	
	Removal	Grass
<i>Litter layer</i>		
Year 1	548 (75) ^a	592 (77) ^a
Year 2	291 (12) ^a	327 (60) ^a
<i>Roots</i>		
Fine (<2 mm)	209 (36) ^a	191 (49) ^a
Coarse (≥ 2 mm)	144 (50) ^a	145 (47) ^a
Total root	353 (85) ^a	336 (93) ^a
<i>Mineral soil</i>		
Fine (<2 mm)	20 695 (3114) ^a	20 281 (1897) ^a
Coarse (≥ 2 mm, <5 mm)	610 (271) ^a	524 (67) ^a
Rock* (≥ 5 mm)	467 (98) ^a	624 (135) ^a
C_{mic}^{\dagger}		
Year 1	1.9 (0.3) ^a	1.9 (0.1) ^a
Year 2	1.9 (0.1) ^a	1.9 (0.5) ^a
Total mineral soil	21 771 (2948) ^a	21 428 (1936) ^a
Total soil carbon	22 627 (2855) ^a	22 356 (1772) ^a

Values are treatment means (± 1 SE; $n = 3$); means with the same letter within a row do not differ at $\alpha = 0.05$.

*Organic matter (roots and mineral soil C) contained within porous lava rocks.

[†]Microbial biomass C ($\text{mg C g dry soil}^{-1}$).

Results

Soil C pools

No differences were observed in any soil C pools between removal and grass plots 3–5 years after grass removal ($P > 0.39$; Table 2). Total soil C was $\sim 22\,500 \text{ g C m}^{-2}$ to 30 cm, and was dominated by the mineral soil fine fraction which accounted for 91% of total soil C in both treatments despite only accounting for 9% of soil mass and $\sim 35\%$ of soil volume. Of the remaining C, 5% was in rocks and the mineral soil coarse fraction, while litter and fine roots accounted for an additional 1–2% each. For pools measured in both years, litter C was $\sim 45\%$ smaller in the wetter Year 2 ($P < 0.03$) and C_{mic} did not differ between years ($P = 0.99$).

Soil-surface CO_2 efflux

Instantaneous measurements of R_{soil} were higher in grass plots ($P < 0.01$; Fig. 1a). Across both treatments R_{soil} ranged from 0.81 to $7.12 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, with overall means of 3.09 and 4.39 in removal and grass plots, respectively. R_{soil} varied with time ($P < 0.01$) and displayed a significant time-by-treatment interaction ($P < 0.01$). Soil temperature was higher in removal plots

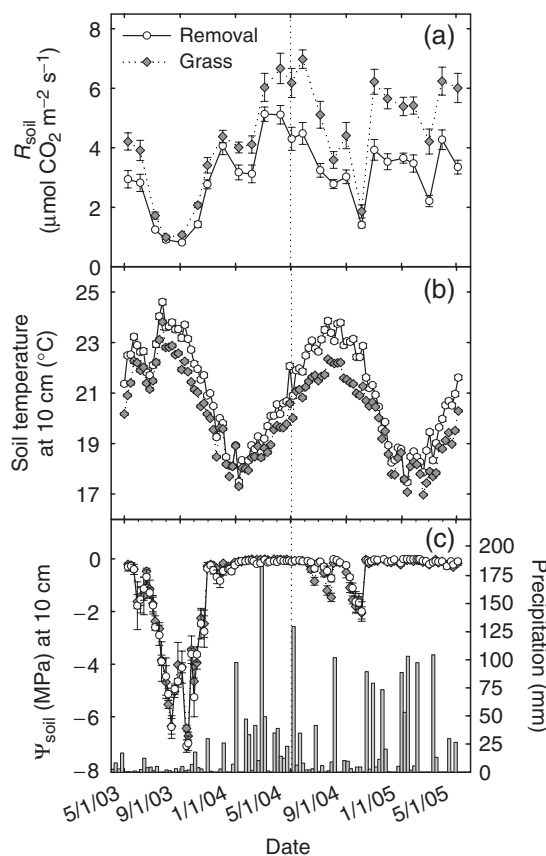


Fig. 1 Two year time course of treatment means (± 1 SE) for (a) monthly soil-surface CO_2 efflux (R_{soil} ; $n = 4$), (b) weekly soil temperature ($n = 3$), and (c) weekly soil water potential (Ψ_{soil} ; $n = 3$) with weekly cumulative precipitation (shaded bars), in grass plots (shaded diamonds and dotted lines) and removal plots (open circles and solid lines). The dotted vertical line in each panel delineates Years 1 and 2 measurements.

($P = 0.01$; Fig. 1b), and there was a distinct temporal pattern to temperature ($P < 0.01$) that differed by treatment ($P = 0.02$). Soil temperature peaked during the summer but varied by only $\sim 5^\circ\text{C}$ throughout the year (Fig. 1b). Soil water potential also varied with time ($P < 0.01$), with driest conditions in the summer, but neither treatment nor time-by-treatment interactions were significant ($P = 0.93$ and 0.69 , respectively; Fig. 1c).

For both grass and removal plots, there was no relationship between R_{soil} and soil temperature at 10 cm ($R^2 = 0.04$; $P > 0.10$), and the slope of the relationship was negative for both treatments. There was also no significant diel fluctuation in R_{soil} for either treatment ($P > 0.50$). For the 24 h measurement period, soil temperature varied by only 2°C and R_{soil} by $< 10\%$ between maximum and minimum measured values.

In contrast, R_{soil} closely followed seasonal patterns of Ψ_{soil} (Fig. 1), although the strong seasonality of both

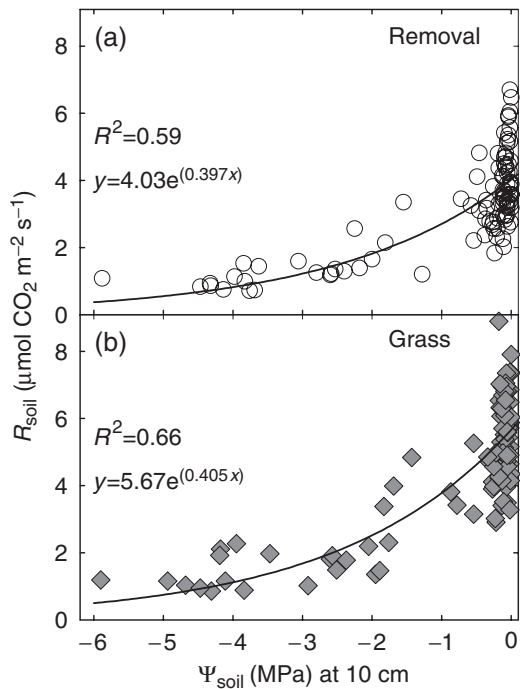


Fig. 2 Soil-surface CO₂ efflux (R_{soil}) was strongly related to soil water potential (Ψ_{soil}) ($P < 0.01$) in both (a) removal plots and (b) grass plots over a wide range of Ψ_{soil} observed during 2 years of measurements.

was diminished in the wetter Year 2. There was a significant correlation between R_{soil} and Ψ_{soil} for both removal and grass plots (Fig. 2). The relationship was best explained by an exponential model, which accounted for 59–66% of the variance in the data. Model fit was best at $\Psi_{\text{soil}} < -0.5$ MPa, as flux rates varied more when soils were at or near saturation. Because flux rates increased with grass invasion, the slope of the relationship between R_{soil} and Ψ_{soil} was higher in grass plots.

R_{soil} responded rapidly to a large precipitation pulse (95 mm), increasing by >75% within 24 h in both treatments (Fig. 3), despite the fact that soils were near saturation (-0.35 MPa) before the pulse event. Cumulative R_{soil} based on linear interpolation between three monthly measurements over a 58-day period (Fig. 3; solid line) resulted in flux estimates that were 18–22% lower than that from linear interpolation between flux measured every 2–3 days (Fig. 3; dashed line). However, when monthly measurements were supplemented by estimating R_{soil} weekly using the flux vs. Ψ_{soil} relationship (Fig. 3; dotted line), cumulative flux differed by <6% from estimates based on intensive measurements every 2–3 days.

Despite the large effect of pulse events on cumulative flux over short temporal scales, there was less difference

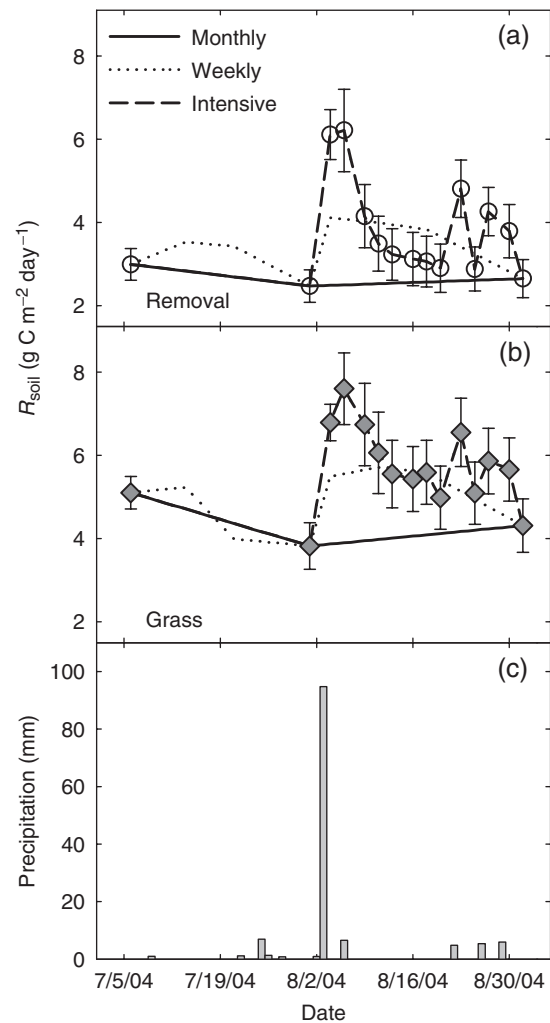


Fig. 3 Daily soil-surface CO₂ efflux (R_{soil} ; g C m⁻² day⁻¹) increased by >75% in both (a) removal plots and (b) grass plots within 3 days of (c) a large precipitation event. Linear interpolation between monthly efflux measurements (monthly; solid line) underestimated cumulative flux over this 58-day period by 18–22% compared with interpolation between measurements taken every 2–3 days (intensive; dashed line). Cumulative flux from intensive measurements differed by 1–6% when linear interpolation of monthly efflux measurements was supplemented with efflux predictions based on weekly soil water potential measurements (weekly; dotted line).

on an annual scale between cumulative flux calculated with interpolation between monthly measurements and that calculated by supplementing monthly measurements with weekly estimates of R_{soil} from Ψ_{soil} (0.5–7%; Table 3). Herein, we use annual cumulative R_{soil} calculated from linear interpolation between monthly measurements supplemented with weekly estimates based on the flux vs. Ψ_{soil} relationship.

Table 3 Cumulative annual flux of C from soils (R_{soil} ; $\text{g C m}^{-2} \text{yr}^{-1}$) in grass and grass removal plots

	Soil-surface CO_2 efflux ($\text{g C m}^{-2} \text{yr}^{-1}$)	
	Removal	Grass
<i>Monthly interpolation</i>		
Year 1	1039 (59) ^a	1318 (73) ^b
Year 2	1322 (83) ^a	1957 (118) ^b
<i>Weekly interpolation</i>		
Year 1	1024 (32) ^a	1417 (24) ^b
Year 2	1384 (24) ^a	1943 (49) ^b

Estimates are based on either linear interpolation between monthly efflux measurements (monthly), or linear interpolation between monthly efflux measurements supplemented with weekly efflux predicted from soil water potential measurements (weekly). Values are treatment means ($\pm 1 \text{SE}$; $n = 4$); means with the same letter within a row do not differ at $\alpha = 0.05$.

Annual cumulative R_{soil} was 38–40% higher in grass plots over the course of the study ($P < 0.01$; Table 3), ranging from a low of $1024 \text{ g C m}^{-2} \text{yr}^{-1}$ in removal plots in Year 1 to a high of $1943 \text{ g C m}^{-2} \text{yr}^{-1}$ in grass plots in Year 2. R_{soil} increased by $\sim 36\%$ in both treatments in the wetter Year 2 ($P < 0.01$). Annual R_{soil} was positively and linearly correlated with total LAI (understorey + overstorey) across all plots and both measurement years (Fig. 4).

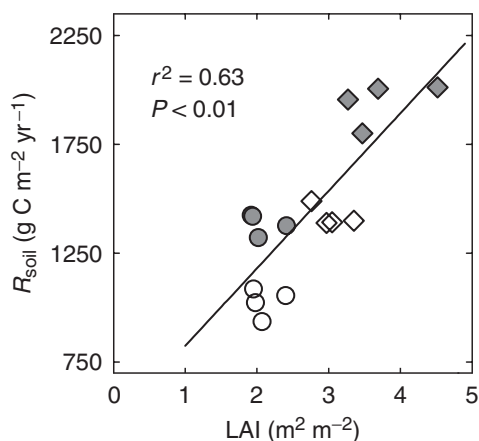


Fig. 4 A positive linear relationship existed between annual soil-surface CO_2 efflux (R_{soil}) and total leaf area index (LAI; overstorey + understorey) across all treatments and years. Values are plot means – circles are removal plots and diamonds are grass plots; open symbols are Year 1 values (normal precipitation) and shaded symbols are Year 2 values (higher precipitation).

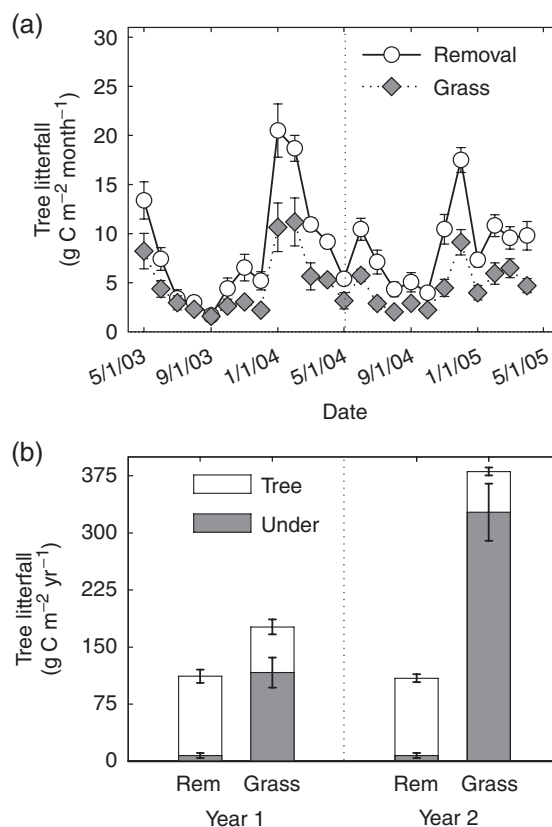


Fig. 5 Two-year time course of (a) monthly tree litterfall (Mean $\pm 1 \text{SE}$) in removal (open circles, solid line) and grass (shaded diamonds, dotted line) plots, and (b) total annual aboveground litterfall (Mean $\pm 1 \text{SE}$) of tree (open) and understorey (shaded) components. Total litterfall and litterfall components all differed significantly by treatment within a given year at $\alpha = 0.05$. The dotted vertical line in each panel delineates Years 1 and 2 measurements.

Aboveground litterfall and litter decomposition

Monthly aboveground tree litterfall was higher in removal plots ($P < 0.01$; Fig. 5a) and displayed distinct seasonality ($P < 0.01$) that differed by treatment ($P = 0.04$). Tree litterfall was lowest during the dry summer (Fig. 5a) and, overall, followed seasonal patterns of R_{soil} (Fig. 1a). Annual aboveground tree litterfall was $\sim 2 \times$ higher in removal plots ($P < 0.01$; Fig. 5b), did not differ between years ($P = 0.40$), and exhibited no time-by-treatment interaction ($P = 0.70$). Understorey litterfall was 16–44 times higher in grass plots ($P < 0.01$; Fig. 5b) and increased by 170% in Year 2 ($P < 0.01$). High understorey litterfall in grass plots more than compensated for reduced tree litterfall in both years such that total aboveground litterfall was higher in grass plots ($P < 0.01$; Fig. 5b), with significant time ($P < 0.01$) and time-by-treatment interactions ($P < 0.01$).

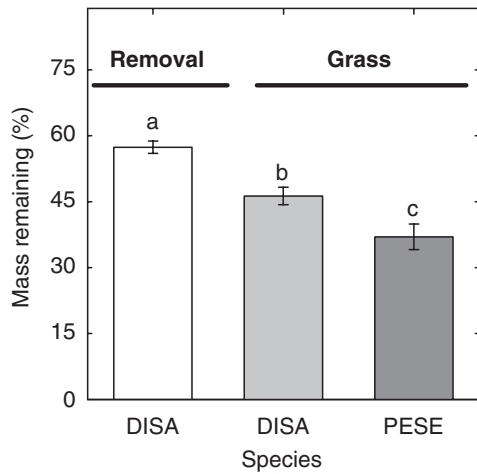


Fig. 6 *In situ* foliage decomposition of the dominant tree (DISA, *Diospyros sandwicensis*) was significantly higher with a non-native invasive grass in the understory (grass) than without (removal). In addition, grass foliage litter (PESE, *Pennisetum setaceum*) decomposed faster than tree litter from either treatment. Bars are treatment and species means (± 1 SE; $n = 4$); means with different letters indicate statistically significant differences at $\alpha = 0.05$ (Tukey's HSD multiple comparison test after one-way ANOVA).

Foliage litter decomposition of *D. sandwicensis*, the dominant native tree, was more rapid in grass plots than removal plots ($P = 0.02$), with 46% and 57% of the original mass remaining after 10 months, respectively (Fig. 6). Treatment differences existed in both microenvironment and initial litter chemistry. In grass plots, litter layer moisture (quantified gravimetrically in March 2005) was $\sim 2 \times$ higher and % lignin was $\sim 25\%$ lower ($P < 0.01$) than in removal plots. In addition, leaf mass per area (LMA) and foliar C:N were higher, and tree leaf N concentration lower in grass plots ($P < 0.03$). Grass foliage in grass plots decomposed faster than tree foliage in both grass and removal plots ($P < 0.02$), with only 37% mass remaining after 10 months (Fig. 6).

*C*₄ contribution to soil C pools and fluxes

Grass contributed substantially to soil C pools for only the litter layer (25–34%) and fine roots (72%) in grass plots (Fig. 7). In removal plots, residual grass material contributed 11% to the fine root C pool (live + dead roots) and $\sim 2\%$ to litter layer C 4 years after removal. There was a negligible contribution of C₄-C to the large mineral soil C pool in both treatments ($< 3\%$; Fig. 7). In contrast, the C₄-C contribution to flux in grass plots was 77% for *R*_{soil}, and 65% and 85% for aboveground litterfall in Years 1 and 2, respectively. In removal plots, there

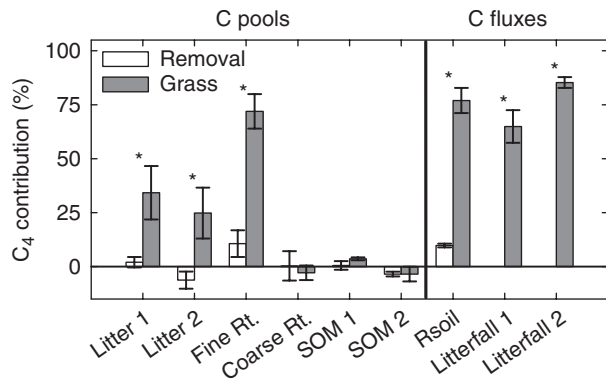


Fig. 7 C₄ grass contribution (%) to soil C pools (left panel) and fluxes (right panel). When significant (*), the C₄ contribution was always greater in grass plots (shaded bars) than in removal plots (open bars). For carbon in the litter layer (litter), mineral soil fine fraction (SOM) and aboveground litterfall (litterfall), data are presented separately for Years 1 and 2. Values are treatment means (± 1 SE; $n = 3$ for pools, $n = 2$ for *R*_{soil} and $n = 4$ for litterfall).

was no C₄ contribution to aboveground litterfall and a small contribution to *R*_{soil} (10%; Fig. 7).

LAI

Overstory LAI was $\sim 40\%$ higher in removal plots than in grass plots ($P = 0.04$; Table 1) and did not vary across years ($P = 0.45$). Both understory and total (understory + overstory) LAI were higher in grass plots ($P < 0.01$) and increased in the wetter Year 2 of the study ($P = 0.01$), due exclusively to changes in understory LAI.

Discussion

Previous studies have shown that invasive grasses can impact soil nutrient (Mack, 2003; Mack & D'Antonio, 2003) and plant water dynamics (Ludwig *et al.*, 2004). However, studies of invasive species' impacts on belowground C cycling have been few, have primarily dealt with C pools, and have varied widely in the magnitude and direction of inferred impacts (Scott *et al.*, 2001; Ehrenfeld, 2003; Hook *et al.*, 2004). Grass invasion into this forest greatly increased the flux of C into and out of soils, as well as the composition of rapidly cycling C pools. However, minimal grass-derived C has been stabilized in soil organic matter, and after ≥ 50 years in the system grass invasion has not altered the size or isotopic signature of the mineral soil C pool.

Soil C pools and C₄ contribution

Our estimate of total soil C in this system is $\sim 4 \times$ larger than C in aboveground live biomass ($\sim 55 \text{ Mg C ha}^{-1}$; Litton *et al.*, 2006) and, thus, represents a very large and

important pool of ecosystem C. In agreement with our first hypothesis (H1), invasion by a C₄ perennial bunchgrass has not led to changes in the overall size of soil C pools even though non-native grass has been a dominant component of the understory for ≥ 50 years. Previous studies have also shown little to no impact of invaders on soil C pools (Ehrenfeld, 2003; Hook *et al.*, 2004). We also hypothesized that the lack of change in soil C pools would be a function of new grass-derived C₄-C replacing the net loss of forest C₃-C following invasion, as seen in other studies dealing with conversion of forests to pasture and cropland (Osher *et al.*, 2003) and reforestation of abandoned cropland (Bashkin & Binkley, 1998; Binkley & Resh, 1999). Such replacement was evident in our study for the litter layer and fine root C pools, where net increases in C₄-C compensated for net losses of C₃-C (Fig. 7). However, these two components represent a small fraction of the total soil C pool. In contrast, for the very large mineral soil fine fraction pool, we found essentially no C₄-C (Fig. 7), suggesting that either belowground inputs of C₄-C are too small relative to the total mineral soil C pool or that C₄-C inputs are primarily to rapidly cycling pools that preclude stabilization in soil organic matter.

Differences between our results and prior studies (Bashkin & Binkley, 1998; Binkley & Resh, 1999; Osher *et al.*, 2003) are likely due to differences in soils and the scope of land cover change encompassed in each study. Prior studies were conducted on deep ash soils with high clay content, while soils in this study contained little clay and $>2\times$ the amount of organic C. In addition, this study examined the impact of grass invasion into intact forests, while prior studies dealt with more pronounced changes in land cover (e.g. forest to cropland).

Soil C fluxes and C₄ contribution

In agreement with the second hypothesis (H2), R_{soil} was higher in grass-invaded plots (Fig. 1, Table 3). Increased R_{soil} in grass plots was related to changes in total stand LAI (tree + understory) following invasion (Fig. 4). This result corroborates the increasingly common finding that R_{soil} is tightly linked to canopy photosynthetic activity (Campbell *et al.*, 2004; Irvine *et al.*, 2005). We attribute higher R_{soil} in grass-invaded plots to both greater LAI (Table 1) and enhanced litter decomposition (Fig. 6). Increased LAI would enhance the photosynthetic capacity of the system, while faster decomposition would shorten mean turnover time of soil C.

Our measurements of C fluxes into and out of soils do not take into account C that is allocated belowground by plants (total belowground C flux, or TBCF; Litton *et al.*, 2007), but because R_{soil} is the dominant compo-

nent of the mass balance equation for estimating TBCF (Giardina & Ryan, 2002; Litton *et al.*, 2004), increased R_{soil} indicates that more C is allocated belowground in grass plots than in removal plots. However, our data also indicate that the potential increase of TBCF with invasion has not led to increased soil C storage. Instead, it appears that the majority of C allocated belowground in grass-invaded plots is rapidly returned to the atmosphere as R_{soil} because: (i) grass-derived litter decomposes very rapidly (Fig. 6); (ii) grass-derived litter and mineral soil C are not accumulating (Table 2; Fig. 7); and (iii) R_{soil} is composed largely of C₄-C where grasses occur ($>75\%$; Fig. 7). Grass litter simply appears to decompose too rapidly and completely to be stabilized in mineral soil. In addition, we found no effect of grass invasion on the size of any soil C pool despite higher litterfall inputs, litter decomposition, and, presumably, belowground C flux in grass plots. However, it would likely take a very large change in C inputs to elicit a short-term response in pools (Smith, 2004).

As in previous studies (Yuste *et al.*, 2003; Vasconcelos *et al.*, 2004; Irvine *et al.*, 2005), discrete precipitation pulses greatly increased R_{soil} within 24 h (Fig. 3). Because efflux continued to increase for >72 h after the pulse event, we attribute the high efflux rates following precipitation to enhanced microbial and root activity rather than flushing of CO₂ from soil air pores. Prior studies have shown strong and sustained increases in soil microbial activity and soil CO₂ release following pulse precipitation events (e.g. Miller *et al.*, 2005).

Ignoring the effects of pulse events on cumulative flux calculated from linear interpolation between monthly measurements in this study resulted in large underestimates of efflux over 30–60 days. In contrast, ignoring discrete pulses had a much smaller impact on annual R_{soil} estimates (Table 3). Borken *et al.* (2002) also found that R_{soil} responded rapidly to soil rewetting in forest ecosystems, and that the transient increase in efflux was a small portion of annual R_{soil} . In contrast, other studies have shown that increased flux of R_{soil} following pulse events can account for a significant portion of annual soil CO₂ efflux (Lee *et al.*, 2004; Misson *et al.*, 2006).

Contrary to the second part of H2, that the grass contribution to soil C fluxes would be proportional to its contribution to ecosystem C pools, we found that R_{soil} was primarily of C₄ origin in grass plots (77%) despite grasses accounting for a small percentage of ecosystem C ($\sim 5\%$; Litton *et al.*, 2006). Interestingly, our 77% estimate of C₄ contribution to R_{soil} closely matches the fraction of fine roots attributed to grasses (72%) and the C₄ contribution to aboveground litterfall in grass plots averaged over both years (75%). We interpret this as further evidence that grass-derived soil C inputs are

rapidly cycled and returned to the atmosphere. We also found that there was a small C_4 contribution to R_{soil} in removal plots ($\sim 10\%$; Fig. 7). The C_4 isotopic signature of R_{soil} in removal plots may be related to residual root C_4 -C (Fig. 7). However, 10% is close to the overall resolution of the method, and designation of end member values and estimation errors associated with the Keeling plot method could account for most, if not all, of this value in removal plots.

Our estimate of the C_4 contribution to R_{soil} was also based on only one measurement period when Ψ_{soil} was -1.25 MPa. This may not coincide with the C_4 contribution to cumulative annual R_{soil} as the $\delta^{13}\text{C}$ signature of R_{soil} appears to be sensitive to soil moisture (Pregitzer *et al.*, 2006) and Ψ_{soil} varied from -0.01 to -7.11 MPa in this study. Still, the grass contribution to R_{soil} was substantial despite the fact that grass is a very small fraction of ecosystem C.

Variability in precipitation

Considerable uncertainty resides in predictions of future tropical precipitation regimes, with models predicting both increased and decreased rainfall over the next 100 years for different parts of the equatorial Pacific (Meehl *et al.*, 2005; Neelin *et al.*, 2006). It is, thus, important to understand the impacts of changes in precipitation on belowground C dynamics. In line with our third hypothesis (H3), we found that increased precipitation enhanced the flux of C into and out of soils. However, we also hypothesized that higher precipitation would increase flux across all components and that the increase in flux would be relatively higher in grass plots. In contrast, we found that increased precipitation did not alter tree litterfall (Fig. 5), and that the relative increase in R_{soil} was the same for both treatments ($\sim 36\%$; Table 3). Grass litterfall, on the other hand, increased by 170% with higher precipitation (Fig. 5), increasing the C_4 contribution to total aboveground litterfall from 65% in Year 1 to 85% in Year 2 (Fig. 7). Still, the litter layer C pool was smaller in Year 2, suggesting that decomposition in this system is primarily limited by moisture availability. Together these results indicate that R_{soil} will increase and continue to be dominated by C_4 -C in grass-invaded forests if precipitation increases.

Generality of results

How might these results be influenced by the use of removal plots as a proxy of preinvasion conditions? Díaz *et al.* (2003) characterized the removal effect (i.e. the effect of removing an organism on a measured variable) as consisting of three facets: (i) how the system

works when a particular organism is absent, (ii) response of remaining organisms, or colonizing organisms, to the removal, and (iii) the effect of the actual removal itself on physical, chemical and biotic components of the ecosystem ('removal artifact'). The first two components of the removal effect were directly examined in this experiment – that is, how the suite of native species responded to and how ecosystem variables changed when an invasive grass was no longer present in the system. The third component of the removal effect was minimized in this study – the removal consisted of application of grass-specific herbicide, weed whacking, and manual extraction of aboveground grass biomass. Removal had no physical impact on the soil surface and, importantly, it occurred 3 years before the initiation of the experiment in a tropical system with year-round growing conditions.

What was the composition of the native understory before grass invasion, and what was its potential contribution to belowground C cycling? Very little is known about the native understory composition and structure in this system. Some insight, however, can be gained from an adjacent forest where *P. setaceum* has been removed for > 10 years (Cabin *et al.*, 2000). In this site, native shrubs, vines and herbaceous species have recolonized the understory. These are the same species that have colonized the removal plots. Moreover, the understory at a nearby forest fragment that has never been heavily invaded by *P. setaceum* is very similar in composition and structure to that in our removal plots (Litton *et al.*, 2006).

Management and conservation implications

Future changes in global land cover and land use are uncertain, but are nonetheless predicted to have a large impact on the terrestrial C balance (Levy *et al.*, 2004). In Hawaii, for example, most tropical dry forest has already been converted to non-native grassland (Brueggemann, 1996). In addition, once forests are invaded by *P. setaceum*, natural regeneration of native species all but ceases, indicating that even remaining forest fragments will eventually be converted (Litton *et al.*, 2006). What happens to belowground C cycling following conversion of forests to grasslands? Given the large pools of soil C in this system, small changes in storage following conversion could have large implications for the regional flux of CO_2 to the atmosphere. Prior studies in this system have shown that elimination of the forest canopy and conversion to non-native grasslands in the presence of grazing reduces soil C pools (Elmore & Asner, 2006). Several lines of evidence support a strong role of grass invasion in this reduction of soil C. First, we found virtually no stabilization of grass C in soil

organic matter in invaded plots. Second, R_{soil} in converted grasslands dominated by *P. setaceum* is up to 25% higher than in uninvaded forests (K. Zimmerman & C. M. Litton, unpublished data). Third, removal of the forest overstory will cease C_3 inputs of C into soils. Thus, high C flux rates out of soils in the absence of inputs into long-lived soil organic matter pools (e.g. wood) could explain the loss of soil C following conversion to non-native grasslands documented by Elmore & Asner (2006).

Predicting the impacts of invasive species on belowground C cycling is important for management and conservation globally, as soils are the largest sink and the second largest flux of C in terrestrial ecosystems. Invasive species are already widespread, recent invasions will likely increase in coverage, and new invasions will undoubtedly occur. While this is certainly a challenging prospect, R_{soil} is a good overall indicator of soil C dynamics. It is the dominant component of ecosystem respiration (Bolstad *et al.*, 2004; Curtis *et al.*, 2005; Yuste *et al.*, 2005), and can be the ultimate determinant of the overall C balance in terrestrial ecosystems (Valentini *et al.*, 2000). In this study, there was a strong positive correlation between annual R_{soil} and total LAI, supporting a growing body of studies highlighting the importance of canopy processes in driving R_{soil} (Ekblad & Högberg, 2001; Högberg *et al.*, 2001; Edwards *et al.*, 2004; Ekblad *et al.*, 2005; Tang *et al.*, 2005). This suggests that total stand LAI (overstory + understory), a commonly measured attribute of forest ecosystems, could be a useful indicator for assessing the impacts of invasive species on the most important flux of C from forests. Such an indicator would be particularly useful where estimates of changes in soil C pools are also available.

Conclusions

Understanding the impacts of invasive plant species on C cycling is crucial for predicting how invasions will impact the flux of C across the soil–plant–atmosphere interface and, thus, future atmospheric composition and climate. Earlier studies have primarily attributed changes in belowground C cycling following invasion to increased biomass (Ehrenfeld, 2003). Grass invasion into a forested ecosystem, then, would be expected to have negligible impacts on soil C pools and fluxes owing to its low biomass relative to total forest biomass. To the contrary, we found that grass invasion into a Hawaiian tropical dry forest substantially enhanced the flux of C into and out of soils. However, we observed negligible stabilization of grass-derived C in soil organic matter, and increased soil C fluxes did not change the size of any soil C pool. Greatly elevated rates of R_{soil}

with grass invasion are of particular concern given the importance of this flux in determining overall ecosystem C balance. If invasion ultimately leads to the conversion of forests to grasslands (Litton *et al.*, 2006) and conversion and grazing lead to long-term decreases in soil C storage (Elmore & Asner, 2006), then non-native grass invasion into the understory of this forest could eventually release a large amount of sequestered soil C to the atmosphere. Regardless, it is clear that while grass invasion into forested areas in this system greatly increases soil C flux, as long as the forest canopy remains intact loss of soil C is unlikely.

Acknowledgements

Support for this study was provided by the National Science Foundation (NSF-DEB-0129326) and the USDA Forest Service, Pacific Southwest Research Station, Institute of Pacific Islands Forestry (Research Joint Venture 06-CA-11272177-006). We would like to thank Roger Harris (Hiluhilu Development LLC) and the North Kona Dry Forest Working Group for access to research sites. Colleen Cole, Kenia Melgar, Bham Paritosh, Carlos Torres, Kolea Zimmerman and numerous students from the Department of Biological Science, California State University Fullerton provided field and laboratory assistance during the project. Peter D. Stahl and Lachlan Ingram (Department of Renewable Resources, University of Wyoming) generously provided assistance and laboratory facilities for the extraction and analysis of microbial biomass. We thank Christian Giardina, Jarrod Thaxton, Kate Bradley and two anonymous reviewers for helpful comments on an earlier version of this manuscript.

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