BIODIVERSITY RESEARCH

Influence of an invasive ant on grazing and detrital communities and nutrient fluxes in a tropical forest

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ABSTRACT

Aim Pathways linking grazing and detrital subsystems of terrestrial ecosystems are important for ecosystem processes and function, but remain poorly understood. The invasion of a generalist predator creates a unique opportunity to study the effects of predation across these subsystems. We examine here, the effects of a non-native generalist predator, the little red fire ant (*Wasmannia auropunctata*, Roger) on both grazing and detrital invertebrate communities and ecosystem processes in a rain forest understorey.

Location Gamba Protected Area Complex, south-western Gabon, Africa.

Methods We measured abundances and diversities of understorey grazing and detrital invertebrate communities, soil nutrients, herbivory, litter fragmentation rates and leaf chemistry of a dominant understorey shrub inside and outside of 19 separate invasion fronts. We then explored possible trophic cascades and pathways of interaction using path analysis.

Results Results suggest that invasive ants may alter herbivory regimes, grazing and detrital communities, and may indirectly alter litter decomposition and nutrient cycling in the soil by suppressing important microbivore and detritivore populations with consequences for leaf chemistry.

Main conclusions These results demonstrate that generalist predators may be major drivers of both grazing and detrital subsystems by inducing strong shifts in adjacent communities that ultimately affect ecosystem processes.

Keywords
Biological invasion, ecosystem function, food web, invasive ants, nutrient cycling, trophic cascade.

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INTRODUCTION

Trophic interactions are important features of terrestrial ecosystems affecting grazing and detrital food webs and ecosystem processes (Lawrence & Wise, 2000; Schmitz *et al.*, 2000; Wardle *et al.*, 2005; Fukami *et al.*, 2006; Dunham, 2008); however, pathways linking these subsystems have long been ignored. Detrital systems and their processes had been assumed to be primarily donor-controlled (via resource availability) (Begon *et al.*, 1986; Strong, 1992), but empirical work has led to a growing awareness that drivers of this system are likely to be much more complex (Setälä *et al.*, 1998) and that generalist predators may form an important link between above- and below-ground terrestrial subsystems (Halaj & Wise, 2001; Scheu, 2001; Miyashita *et al.*, 2003; Dunham, 2008). However,

little attention has been paid to the trophic impacts of generalist predators as potential drivers of nutrient cycling or in altering linkages between grazing and detrital subsystems (i.e. communities based on plant versus detrital resources) (Wardle *et al.*, 2005; Fukami *et al.*, 2006). Thus, a better understanding of linkages between these subsystems has recently become a priority for further development of terrestrial ecology (Wardle *et al.*, 2004).

The invasion by a generalist predator creates a unique opportunity for studying the effects predation has across subsystem boundaries. Here, we examined the effects of the little red fire ant (*Wasmannia auropunctata*, Roger), on both grazing and detrital invertebrate communities and ecosystem processes in the rain forest understorey of Gabon. Ants that function as generalist predators often feed on consumers of

both grazing and detrital food webs and thus have a great potential to impact both systems (Holway *et al.*, 2002). Ants also make up an average of 10–20% of the terrestrial animal biomass and up to 25% or more in tropical regions (Schultz, 2000), thus forming a dominant and critical part of terrestrial ecosystems. Although ant invasions can have major direct and indirect effects on ecosystems through competition, predation, seed dispersal, mutualistic interactions and mound formation (Folgarait, 1998; Christian, 2001; Holway *et al.*, 2002; Suarez & Case, 2002), little is known about the indirect trophic effects of ants on ecosystem processes such as nutrient cycling. The impact of ants on soil nutrient regimes has previously focused on direct effects of mound building in soil dwelling ants (Folgarait, 1998); however, ants are also important predators and consumers of detritivores (Porter & Savignano, 1990; Human & Gordon, 1997). Thus, efficient predators such as *W. auropunctata* may drive important changes in nutrient cycling by altering detritivore densities.

The little red fire ant is omnivorous, functioning as a generalist predator as well as a consumer of honeydew and nectar and is native to Central and South America (Le Breton *et al.*, 2005). In recent years, it has emerged as a major exotic pest (Lowe *et al.*, 2000) that is suspected of being in the early phases of a pan-tropical explosion, having been introduced to Africa, Australia, Papua New Guinea, as well as many Pacific archipelagos (Wetterer & Porter, 2003). While generally not a dominant species in its native range, the little fire ant commonly shows exploding population growth in introduced areas and has been described as forming a 'living blanket of ants that kill and eat nearly all other ants in their path' (Hölldobler & Wilson, 1994, p. 63). The combination of aggressive/predaceous nature and explosive populations in invaded areas suggests that they may have profound impacts on invertebrate communities and ecosystem processes, such as herbivory and nutrient cycling as well.

Our aim was to assess how a tropical rain forest understorey community has responded to the invasion of an aggressive, generalist predator and to explore the possible mechanistic pathways of interaction between grazing and detrital subsystems. To do this, we traced interactions across invertebrate communities and indirect interactions with soil nutrient levels, litter decomposition, leaf chemistry and herbivory regimes in areas invaded and not invaded by *W. auropunctata,* using either side of 19 independent invasion fronts.

METHODS

Study site

Work was conducted within the Rabi oil concession of Shell Gabon (1.19° S, 9.86° E), which is located in mature lowland rain forest of the Gamba Protected Area Complex, southwestern Gabon. The concession was selectively logged 18–21 years ago. Other than current petroleum extraction, human activity is strictly limited by Shell Gabon, resulting in a relatively intact faunal community (Lee *et al.*, 2006). The concession is made up of a series of multiple small forest clearings resulting from the creation of laterite platforms for oil drilling that were *c.* 100 m × 100 m, with some variation in size. The vegetation within the forest edge of these platforms is dominated by native woody pioneer species, predominantly *Harungana madagascariensis* and some *Lophira alata*.

Following platform construction, the actual drilling and extraction has relatively minor physical effects on surrounding habitat. However, at Rabi, the new materials are brought in temporarily from a heavily infested and centralized storage yard during drilling operations and appear to be the principle mode of introduction of *W. auropunctata* (Mikheyev *et al.*, 2008). The unrestricted transport of materials among the heavily infested site and un-infested sites during drilling has been determined to be the most probable method of spread at the site. Indeed, the probability of establishment of ants at each platform has been determined to be an increasing function of the number of rig visits to the site, independent of platform age (Mikheyev *et al.*, 2008). Invasion fronts of *W. auropunctata* have been shown to advance slowly, only on the scale of tens of metres per year (Walsh *et al.*, 2004; Walker, 2006; A.S.M. unpublished). Therefore, we assumed this to be the reason why some platforms at Rabi were not completely infested around their entire perimeter despite no obvious differences in vegetation communities and structure, soil, moisture or other abiotic factors, and given the apparent ability of *W. auropunctata* to spread in a diverse array of habitat types and disturbance levels as observed in Rabi. Differential invasion success between platforms was also not likely because of genetic differences in the introduced ants, as most of the infestations within the petroleum concession were caused by the introduction of a single clone of this facultatively clonal species (Mikheyev *et al.*, 2009).

While the invasion of *W. auropunctata* at Rabi also extends beyond edge habitat into the interior forest, the network of infested platforms in Rabi, which are constructed in an identical fashion using laterite soil from a common source and with the same community of surrounding pioneer plants, provided a unique opportunity to study a series of separate invasion fronts (invaded and non-invaded areas) of similar habitat type and disturbance.

Platforms were chosen that had incomplete invasions, such that the invasion did not yet extend to cover all of the platform edge. In this way, comparisons could be made on either side of the front and equidistant to the edge of the platform to maintain similarity in habitat type, structure and disturbance regime. All of our analyses were restricted to edge habitat to ensure similarity between invaded sites and non-invaded sites. The spread of *W. auropunctata* within the oil concession provided us with a 'natural' experiment of ant invasion and a sample size of 19 separate invasion fronts. We set up paired sites in forest edge habitat on either side of the invasion fronts. We selected sites on the basis of previous surveys, which revealed platforms with incomplete invasion (infestation that did not surround the entire perimeter of the platform) (Mikheyev *et al.*, 2008). To establish the extent of each

invasion we walked the perimeter of each platform examining vegetation, leaf litter and extra-floral nectaries of plants in the forest edge habitat. When an invasion centre was determined, we walked 10 m perpendicular to the platform edge into the forest where our sampling would take place. We then located the nearest *H. madagascariensis* plant (dominant vegetation of all platform edge habitats). This plant was marked and then a flag was placed on each corner of an imaginary 2 m × 2 m square surrounding the plant. Winkler sampling, sticky trap sampling, pitfall trapping and litter bag sampling were designated, in that order, to each of the four flagged corners. We then identified the approximate centre of the non-invaded area on the perimeter of the platform. We sifted leaf litter and soil, searched potential nesting areas and did another visual inspection of vegetation, to ensure the site was not infested. From the centre of the non-invaded periphery of the clearing, we then walked 10 m perpendicular to the platform edge into the forest. We checked the area again for invasion. When no invasion was found, we located the nearest *Harungana* plant and repeated our sampling set up described above.

All of the paired sites had the same soil type and similar vegetation community and structure. The paired sites were located in the forest equidistant from the forest edge (10 m) and always uphill from the laterite platform. While not as ideal as an experimental introduction to assess impacts of an invasive species (which would have carried ethical concerns in this protected rain forest environment), this system of repeated invasions allowed us to avoid pseudo-replication by providing a sample size of 19 independent invasion fronts. For reasons of logistic limitations, we sacrificed within-site replication to maximize replication on the level of independent invasion fronts. More power is gained for testing hypotheses by having a larger number of replicate paired sites but lacking within-site replication, compared with having a small number of replicate paired sites and high within-site replication (Hurlbert, 1984).

Forest floor invertebrate surveys

In February, 2007, we conducted surveys within the paired sites to examine invertebrate abundance and diversity of the forest floor. We used three methods for surveying invertebrates including (1) pitfall traps, (2) mini-Winkler extraction, and (3) sticky traps. Sampling was performed in the layout described previously, 10 m into the forest from the platform clearing.

Pitfall traps were used to survey macroinvertebrates (> 5 mm) of the forest floor. A trap was placed in each site ($n = 38$) and run for 5 days before being collected. For each trap, we sunk a 500-mL plastic cup with an upper diameter of 8.8-cm flush with the surface of the ground. Three cm below the rim, a series of pin holes were made to prevent overflow during heavy rain. Each trap was filled with *c.* 120 mL solution (1:4) of ethanol and water plus a drop of dish detergent. Tops were protected from rain with 15 × 15 cm roofs constructed from black plastic sheeting material. Four drift fences measuring 0.75 m in length were created with large fallen leaves (propped up to create a fence with wooden stakes) and

radiated from four sides of the pitfall trap to potentially increase trapping success (Winder *et al.*, 2001). After 5 days, traps were collected and invertebrates were placed in an ethanol solution for later identification.

In each site, litter was collected from a 1-m² area and sifted to remove large debris before being placed in mini-Winkler extraction sacks for 2 days to extract invertebrates (following Fisher, 1999). Winkler samples from two plots were lost during transport back to the USA and are thus absent from the analysis (reducing the sample size to 17).

Sticky traps placed horizontally on the surface of the forest floor were employed for additional sampling microinvertebrates in each paired site (see Dunham, 2008, for discussion of method). To make the traps, a thin layer of Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI, USA) was spread across a clear acrylic plate (5 × 10 cm) with rows of pre-marked 1 × 1 cm squares to facilitate counting. Sticky traps were placed level with the leaf litter and sheltered from falling debris and rainfall with a roof made of black plastic sheeting. One trap was placed in each site until day 5 when the traps were collected and microinvertebrates were identified with the aid of a stereomicroscope. One site was removed from analysis because a trap was carried into a burrow by a land crab.

Herbivores and herbivory

To examine effects of invasive ants on herbivory rates, we used a shrub of *H. madagascariensis* marked at each site (as described previously). Four branches were chosen systematically on each plant as arising from branch nodes 1–4 from the base of the plant. Cuttings were then made from the fifth leaf internode from the outermost end of the branch. Once cut, the branches were immediately bagged and taken to the lab for analysis. Branches and bag contents were placed on a white plastic viewing tray and examined visually for invertebrates, which were then removed, counted and identified to family and trophic guild. Each leaf was then examined under a stereomicroscope to count and identify less mobile invertebrates such as mites, aphids and scales. The branches were then used to estimate percentage leaf tissue lost to herbivory. Each branch was assigned an estimated herbivory level of 1–4 (1: 0–5%, 2: 5–10%, 3: 10–20%, 4: > 20%). Leaf herbivory, expressed as a percentage of the leaf area missing, has advantages over absolute leaf area measures because holes left by herbivory expand proportionally as the leaf grows and because it also allows branches containing different total leaf areas to be compared (Reichle *et al.*, 1973).

Litter bags

Litter bags are the most commonly used technique for examining litter decomposition in terrestrial ecosystems (Wider & Lang, 1982; Graça *et al.*, 2007). A litter bag was placed in each paired site to examine the impact of *Wasmannia* invasion on breakdown of leaves through fragmentation and decomposition. The litterbags (10 × 10 cm) were made of

nylon material, with 2-mm mesh to allow invertebrate access. Litterbags were filled with 2.5 g of naturally senesced leaves collected under a single *H. madagascariensis* tree located in an uninvaded area separate from all platform sites and dried to a constant weight. After 20 days in the paired sites, litter bags were collected and litter was dried, carefully cleaned of debris (roots, soil, etc.) and weighed to the nearest 0.01 g. One litter bag was found damaged from breakdown of mesh material and was removed from analysis.

Soil and leaf nutrients

The two youngest leaves from the apical end of each branch used for herbivore sampling were collected for nutrient analysis. Leaves were dried at 70 °C, ground to a powder and sent to Kansas State Soil Lab for analysis of Carbon, Nitrogen and Phosphorus content.

At the end of the experiment three, 8-cm-diameter soil cores were taken of the first 10 cm of soil in each site within 25 cm from the position of litter bag placement. Samples from each site were mixed, air-dried and sifted to remove large rocks and debris. A 10-cm-diameter circle of leaf litter was also collected at the site of soil sampling for nutrient analysis. Soil and litter samples were sent to Kansas State Soil Testing Laboratory for analysis of total and inorganic nitrogen and phosphorus and carbon. After a KCl extraction of ammonium and nitrate, a cadmium-reduction was applied and levels of both nutrients were determined through colorimetric assays run in separate channels in a flow analyser to measure the ions simultaneously. Inorganic nitrogen was determined from the sum of ammonium and nitrate. The Mehlich-3 technique was used to extract inorganic phosphorus (Mehlich, 1984). To measure total nitrogen and phosphorus levels, a modified Kjeldahl digestion (Parkinson & Allen, 1975) was performed and the diluted digest was analysed in separate colorimetric reactions using a flow analyser. One site was removed from the soil analyses after confusion resulting from errors in transcription of sample labels during lab processing.

Statistical analyses

Effects of ant invasion in paired sites were carried out using student's paired *t*-tests. Log transformations of data were performed when necessary to achieve normality of data. In our multiple comparisons, we corrected for the false discovery rate using a Benjamini–Hochberg procedure to control for type I error (Benjamini and Hochberg 1995). Mechanistic interpretation of these results is difficult, however, because of the multitude of possible indirect effects as a result of invasion. We thus used path analysis (Amos 7.0 software; Amos Development Corporation, Mount Pleasant, SC, USA) to explore cause–effect hypotheses and identify potential indirect effects. Path analysis uses a multivariate system of equations linking dependent and independent variables to partition relationships between variables based on hypothesized pathways of interaction (Wootton, 1994). The path coefficients serve as measures of the 'strength of interaction', an index reflecting the relative amount of variation in a target variable that can be explained by variation in various interaction variables. Ultimately, path analysis deals with correlation and not causation of variables; however, the usefulness of this method in the current study is in translating *a priori* hypotheses concerning the 'causal' links between variables into statistical models capable of falsification. Path analysis can thus provide non-intuitive insights into species interactions and is logistically more feasible than exhaustive pairwise experimental manipulation. It also provides a stronger interpretation than simply employing regression techniques (Wootton, 1994). We used path analysis in our discussion based on results of our experiment to eliminate potential pathways and to explore hypotheses about how ant invasion might have influenced soil nutrient levels and plant chemistry.

RESULTS

Forest floor invertebrate surveys

Pitfall traps revealed lower abundance and diversity of native macroinvertebrates (> 5 mm) on the forest floor in areas that were invaded by *W. auropunctata* (Fig. 1). There were significantly fewer (40%) macroinvertebrates inside *W. auropunctata* invasion fronts based on pitfall trapping (Table 1). Diversity was also affected. Mean family richness of macroinvertebrates per site was significantly lower within the invasion front than outside (Table 1). Average morpho-species richness and a Shannon index of diversity for native macroinvertebrates were also lower within *W. auropunctata* invasion sites; however, the significance of these findings ($P = 0.02$, and $P = 0.03$ respectively) was lost after a Benjamini–Hochberg correction for multiple testing (Table 1).

Abundance of total native microinvertebrates was significantly lower in ant-invaded sites sampled by sticky traps but not with Winkler extraction (Table 1). This difference is probably attributed to biases in sampling techniques as can be seen in Fig. 2. The Winkler extraction sampled proportionally more mites (Acari) (paired *t*-test, $t_{31} = 6.26$, $P > 0.001$) and proportionally fewer Collembola (paired *t*-test, $t_{31} = 11.99$, $P > 0.001$) than sticky trap samples. Abundance of Collembola in ant-invaded areas was found at one half the levels found outside the invasion front in both Winkler samples and sticky traps (Table 1).

Herbivores and leaf chemistry

All *H. madagascariensis* plants evaluated in invaded sites were infested with *W. auropunctata*. Numbers of phloem feeding herbivores were significantly higher and leaf chewing, invertebrate herbivores were significantly lower on plants with *W. auropunctata* than on plants in uninvaded sites with no ants present. For plants in uninvaded sites holding native ants, this same pattern was observed (Fig. 3a), but the significance of both relationships ($P = 0.02$ for both) was lost after
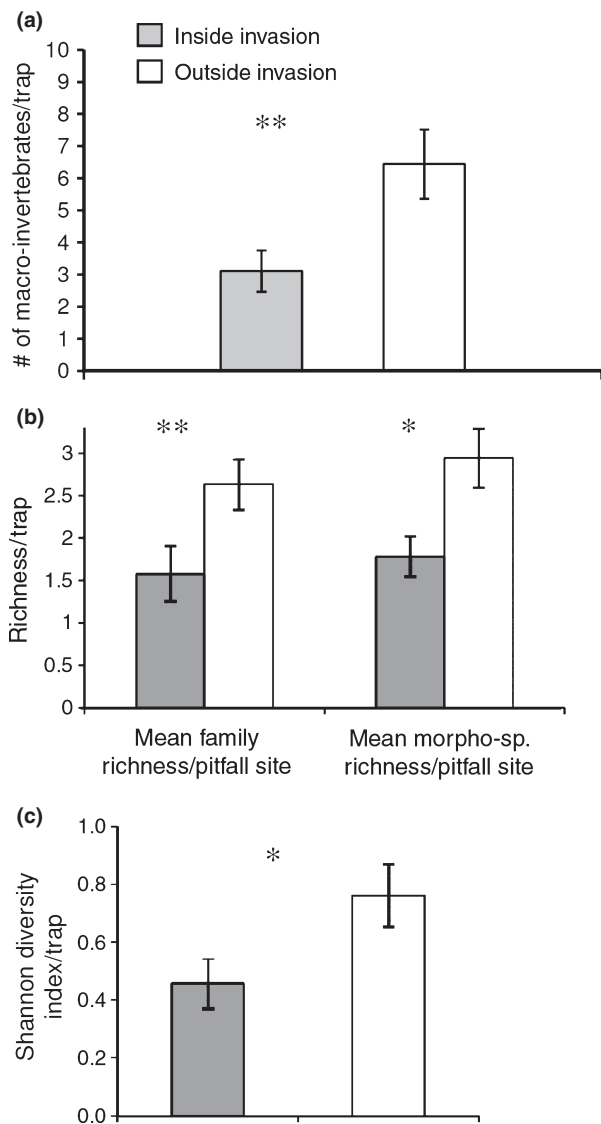
Figure 1 (a) Number, (b) family and morphospecies richness and (c) Shannon species diversity index of macroinvertebrates found with pitfall traps in *Wasmannia auropunctata* invaded sites and non-invaded sites. *$P < 0.05$, **$P < 0.01$.

Table 1 Results of two-tailed Student's *t*-tests describing differences between invaded sites and non-invaded sites for various ecosystem variables.

Variables	Eff-rel	d.f.	t	P-value
Leaf litter invertabrates				
Macroinvertebrates				
Abundance*	–	18	3.18	0.005
Family richness*	–	18	2.72	0.01
Morpho-sp. richness	–	18	2.50	0.02
Shannon Index	–	18	2.36	0.03
Microinvertebrates				
Winkler extraction				
Total abundance	–	15	1.89	0.08
Collembolan abundance*	–	15	3.15	0.007
Sticky trap sampling				
Total abundance*	–	18	3.04	0.007
Collembolan abundance*	–	18	3.13	0.006
Herbivores on plants				
Herbivory (leaf area lost)*	–	18	7.32	0.000
Phloem feeders				
Invaded versus no ants*	+	6	3.97	0.007
Invaded versus native ants	+	11	2.60	0.02
Leaf chewers				
Invaded versus no ants*	–	13	3.45	0.004
Invaded versus native ants	–	4	3.50	0.02
Leaf chemistry				
Total N	–	14	0.10	0.98
Total P	–	14	1.89	0.07
N:P ratio	+	14	2.26	0.04
Soil nutrients				
Total N	–	17	1.35	0.19
Total P	–	17	0.56	0.59
Inorganic N	–	17	0.15	0.88
Inorganic P	–	17	2.00	0.06
Leaf litter				
Litter bag mass loss	–	16	1.75	0.13
Litter C:N	–	18	0.31	0.76
Litter C:P	–	18	0.83	0.42

Eff-rel is effect relationship (+/–).

*Significance after a Benjamini and Hochberg control for false discovery rate for multiple comparisons (Benjamini & Hochberg 1995).

application of a Benjamini–Hochberg correction for multiple testing (Table 1). No plants were observed during the study to hold both native ants and *W. auropunctata*. Invasion was also associated with a reduced amount of leaf area loss by herbivores by an estimated 60% (Fig. 3d). Areas outside the invasion front had a significantly higher degree of herbivory than within the invasion (Table 1).

Analysis of leaf chemistry of *H. madagascariensis* plants in invaded and non-invaded sites revealed no significant difference in leaf nitrogen (Table 1). However, leaf phosphorus levels were significantly lower in *W. auropunctata* infested sites by 11% (Table 1, Fig. 3c). Ratios of N:P in *H. madagascariensis* leaves were 10% higher in *W. auropunctata* infested areas (Table 1).

Soil nutrients and leaf litter

Soil inorganic phosphorus was not significantly lower within ant-invaded sites relative to uninvaded sites (Table 1). However, a significant correlation was found between inorganic phosphorus levels in the soil and Collembolan densities across sites (ant invasion used as covariate, Pearson's $r = 0.45$, $P < 0.02$). There was no difference in total nitrogen levels or total phosphorus levels (Table 1) between invaded sites and non-invaded sites. Inorganic nitrogen (NH_4 and ammonia) also did not differ between invaded sites and non-invaded sites (Table 1). We found no significant differences in litter quality between invaded plots and uninvaded plots using C:N and C:P nutrient ratios as our measure (Table 1).
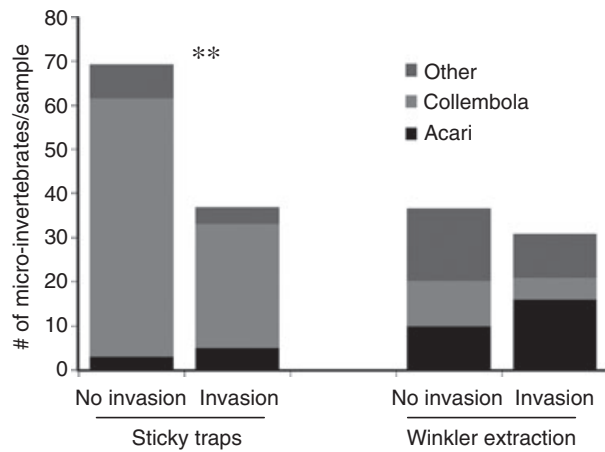
Figure 2 Number of microinvertebrates found through sticky trap and mini-winkler sampling in *Wasmannia auropunctata* invaded sites and non-invaded sites. *$P < 0.05$.

Figure 4 Results of a path analysis evaluating the effects of *Wasmannia auropunctata* invasion on soil inorganic phosphorus and leaf nutrients. Arrows represent direction of interaction. Dashed lines indicate negative effects and solid lines indicate positive effects. The numbers by the arrows represent interaction strengths (path coefficients) and numbers in parentheses represent the proportion of variance not explained by the model (error variance). Grey lines represent non-significant (NS) interactions, which were not included in the final model. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

Loss of mass from leaf litter bags in invaded sites was not significantly different from that in non-invaded sites (Table 1), but did show a significant negative correlation with *W. auropunctata* abundance (Pearson's $r = -0.47$, $P = 0.01$). Leaf mass loss was also correlated with relative abundance of macroinvertebrate detritivores (invasion used as covariate, Pearson's $r = 0.39$, $P < 0.03$) and Collembola (invasion used as covariate, Pearson's $r = 0.48$, $P = 0.01$).

Path analysis

Generally, path analysis provided evidence for direct interactions between ant invasion and invertebrate abundances and indirect interactions with soil inorganic phosphorus, leaf tissue N:P ratios and litter mass loss from litterbags (Figs 4 & 5). Since path analysis was used to explore possible explanations of
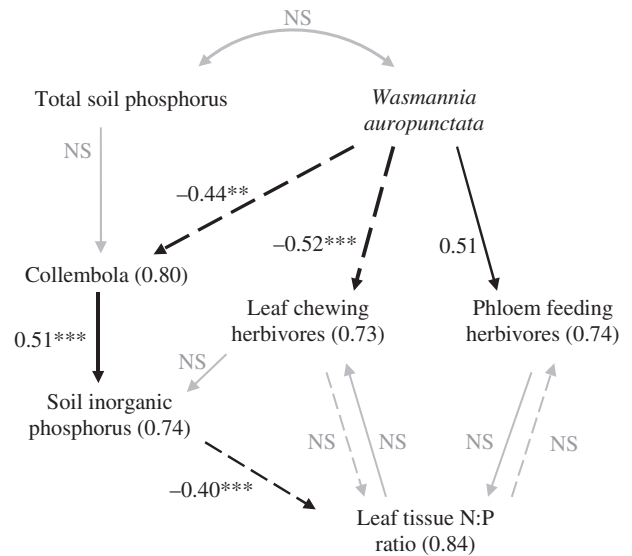
the results described above, the outcome of the analyses is reviewed with more details in the Discussion section.

DISCUSSION

The little red fire ant, *W. auropunctata*, has been gaining in importance as an exotic pest throughout the tropics (Wetterer
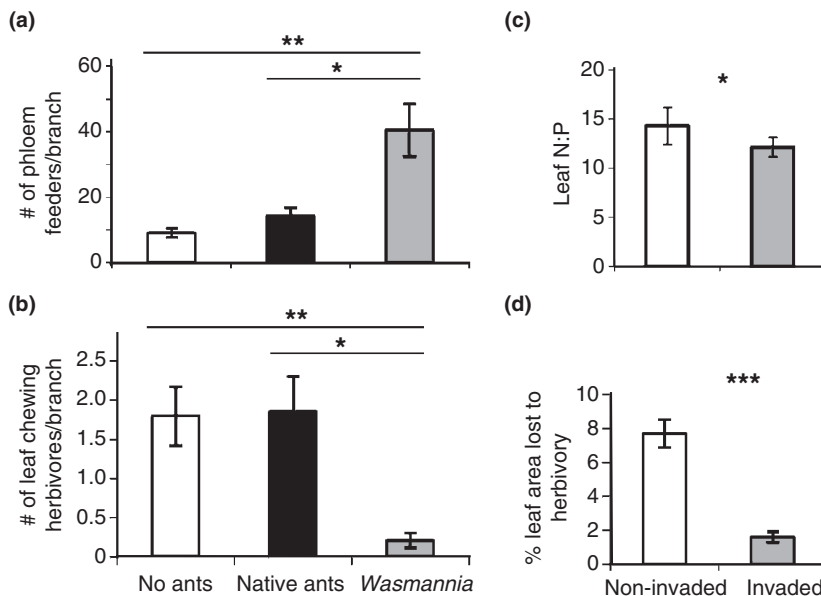

Figure 3 Number of (a) phloem feeding and (b) leaf-chewing herbivores, (c) total phosphorus (p.p.m.) and (d) percentage leaf area lost to herbivory of sampled branches of *Harungana madagascariensis* found in *Wasmannia auropunctata* invaded sites and non-invaded sites. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.
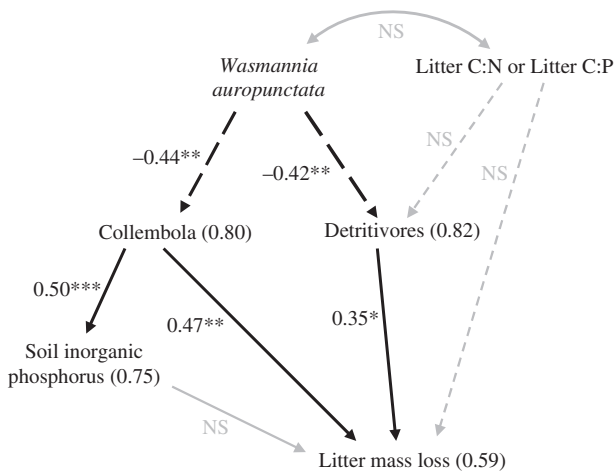
Figure 5 Results of a path analysis evaluating the effects of *Wasmannia auropunctata* invasion on mass loss from leaf litter bags. See Fig. 4 for explanation of symbols. This analysis suggests that trophic links involving detritivores and Collembola may be more important than nutrient levels in explaining the variation in litter mass loss in this study.

& Porter, 2003). At our study site, native macro- and microinvertebrates of the forest floor were lower in both diversity and abundance in areas invaded by *W. auropunctata* with possible links to soil nutrient turnover and litter decomposition. In addition, herbivore communities inhabiting the vegetation in ant-invaded areas appear to have shifted to a system dominated by species of phloem feeding guilds such as aphids and scales.

Our assumption in this study was that the pattern of the invasion resulted from stochastic processes of invasion from the well site and the slow spread rate of invasion from infected rubbish or materials left along forest edge bordering the platforms during drilling operations (Mikheyev *et al.*, 2008). However, the drawback of comparative studies of invasive species, such as ours, is that we cannot definitively rule out the idea that our uninvaded sites are not somehow intrinsically different and more resistant to invasion, despite our attempt to control environmental heterogeneity between paired sites. However, there were several factors providing support that the 'blanket' of invasive ants covering the understorey litter and vegetation in invaded areas of this rain forest have contributed to the results presented in this study, rather than being driven by differing environmental conditions. These include (1) the relatively slow expansion rate of the study species' distribution (Mikheyev *et al.*, 2008), (2) lower prey densities inside the invasion, (3) subsequent invasion of several control sites (after study was completed), (4) large sample size of multiple invasion fronts, (5) similarity in habitat between paired invaded- and control-sites, and (6) their close proximity.

Native invertebrate diversity and abundance

Wasmannia auropunctata is known to be omnivorous, feeding primarily on invertebrates, plant nectaries and honeydew

produced by Hemiptera such as coccid insects (Holway *et al.*, 2002; Wetterer & Porter, 2003). The most obvious explanation for the reduction in forest floor invertebrate abundance and diversity in areas invaded by little red fire ants is that the ants directly suppress invertebrate numbers by predation. Insect parts and bodies were commonly found in nests of the little red fire ants in this study. Despite the small size of the ants (*c.* 1.5 mm), they have a venomous sting that gives them a greater ability to subdue large as well as small invertebrate prey (Holway *et al.*, 2002). The little red fire ant has been blamed for similar reductions of native flying and arboricolous insects in the Galapagos (Lubin, 1984). Other changes in invertebrate communities may be attributed to competition. Two other studies have shown dramatic declines (virtual elimination) in abundance and diversity of native ant species in Gabon rain forest in the presence of *W. auropunctata* invasion (Walker, 2006; Mikheyev *et al.*, 2008). The low densities of chewing herbivores and high densities of honeydew-secreting phloem feeding insects in invaded sites found in our study are also most likely a result of *W. auropunctata* predation and aggression towards leaf-chewing herbivores and protection and care for Hemiptera that produce honeydew (Wetterer & Porter, 2003). Ironically, *W. auropunctata*'s effectiveness at eliminating herbivores is partly responsible for its wide occurrence in Central Africa, as nests were deliberately transported to Cameroonian cacao plantations to control mirid bug infestations (Bruneau de Miré, 1969).

Soil and leaf nutrients

Understanding variation in soil and leaf chemistry and the potential influence of ant invasion requires an analysis of potential links between grazing and detrital systems. Variation in N:P nutrient ratios in the leaves of *H. madagascariensis* might be in response to changes in herbivory regimes or plant-available phosphorus levels in the soil or both. Changes in leaf-chewing and phloem-feeding herbivore abundances can change nutrient ratios in plant leaves through altered allocation of nutrients towards inducible defences (Baldwin & Ohnmeiss, 1994; Ohnmeiss & Baldwin, 1994) or away from herbivore-damaged leaves (Harris, 1974), or through reduced plant uptake or drain of phloem sap (Khattab, 2007). Similarly, herbivores may also alter soil nutrient regimes through herbivore-mediated changes in litter inputs (see Hunter, 2001, for a review) or through altered uptake by host plants (Ticconi & Abel, 2004). Patterns of soil nutrients may also be altered by changes in detrital communities that alter decomposition rates or decrease immobilization of nutrients (e.g. Lawrence & Wise, 2000; Dunham, 2008).

Data taken from invaded sites and non-invaded sites were used in a path analysis to evaluate and eliminate some of these possible scenarios and identify some of the possible direct and indirect pathways of interaction (Figs 4 & 5). Path analysis allowed us to reject the hypotheses that herbivores were driving patterns of soil inorganic phosphorus or leaf chemistry in this study because links between these variables were not significant

(Fig. 4). The scenario most supported by path analysis was one in which *W. auropunctata* invasion results in higher leaf tissue N:P ratios, primarily through top-down trophic effects (or trait-mediated effects) in the detrital web that alter soil chemistry and then feed back to plants (Fig. 4). *Wasmannia auropunctata* are known from other work to prey upon Collembola (Edwards & Edwards, 2006) and in the present study, they were negatively associated with relative Collembolan abundance. Microbivores, such as Collembola can increase microbial production and the release of inorganic phosphorus from microbial tissues (Anderson & Ineson, 1984; Ingham *et al.*, 1985; Mikola & Setala, 1998). Nutrients are released from the microbivores by excretion or with the death of the organism, which can lead to increased mobilization of inorganic phosphorus (plant available form). Our path analysis shows a positive association of Collembolan abundance with inorganic (plant available) phosphorus in the soil and a negative association of soil inorganic phosphorus with leaf N:P ratios. The latter may be a result of increased phosphorus uptake in areas with higher plant-available phosphorus in the soil. Similar indirect cascading effects of predators of Collembola on soil inorganic phosphorus levels have previously been reported in a West African rain forest and were apparent within a month of predator manipulation (Dunham, 2008).

An alternative hypothesis for the pattern of Collembolan densities in this study was that the changes were not driven by ants, but by bottom-up effects of total phosphorus levels in the soil. Phosphorus is a limiting nutrient for many organisms in tropical forests and other studies have found increasing densities of Collembola and other leaf litter invertebrates with increasing total phosphorus levels in the soil (e.g. see Kovac & Miklisova, 1997; McGlynn *et al.*, 2009). However, we found no significant difference in total phosphorus between invaded sites and non-invaded sites (Table 1) and total soil phosphorus was not associated with Collembolan abundance in this study (Fig. 4). As Collembola are known to reduce immobilization of inorganic phosphorus in the soil from microbial populations by releasing it in a form available for plant uptake (Anderson & Ineson, 1984; Ingham *et al.*, 1985; Mikola & Setala, 1998), this may be the most parsimonious explanation for the pattern observed in this study. However, further mechanistic studies may be necessary given the comparative nature of our study.

Litter bag mass loss

The negative correlation of relative *W. auropunctata* abundance and mass loss from litter bags observed in the study was hypothesized to be either an indirect effect of changes in detrital invertebrates or a result of differing quality of surrounding litter, which may alter microbial activity. Detritivores are important for the initial break down of litter material and suppression of these organisms during ant invasion may have cascading impacts affecting decomposition. Additionally, litter quality as measured by C:N and C:P nutrient ratios, has also been shown to play a major role in the rate of litter decomposition (Schlesinger, 1997). Path analysis

of our data supported the hypothesis that litter mass loss was negatively affected by a top-down cascade of effects involving trophic and/or trait-mediated effects of *W. auropunctata* on Collembola and litter consuming detritivores (explaining 41% of the variation in litter mass loss) (Fig. 5). Nutrient ratios (C:N and C:P) of surrounding litter and levels of soil inorganic phosphorus, however, were not significant predictors of litter mass loss (Fig. 5), drawing no support for the alternative bottom-up explanation of our data.

CONCLUSIONS

The results presented here suggest that addition of a dominant generalist predator to a complex terrestrial ecosystem may result in shifts in native diversity, communities and ecosystem processes and may alter effects linking grazing and detrital systems. Evidence suggests that the differences we observed in invaded sites of the complex tropical forest may have occurred through a combination of the ants' mutualistic interactions with phloem feeding insects (and predation or behaviourally mediated impacts on leaf consumers), and intense predation on leaf litter invertebrate communities. By suppressing important microbivore and detritivore populations, they may indirectly alter litter decomposition and nutrient cycling in the soil that leads to altered nutrient levels present in plant tissue. Further research is necessary to determine if this is maintained in the system or is a temporary shift of initial invasion and population expansion.

Implications of results

Few studies have explored the relationships between the presence of invasive ants and whole invertebrate communities and none that we are aware of has examined potential trophic cascades affecting nutrient regimes in both soil and plants. However, studies of two other highly invasive ant species, *Solenopsis invicta* (Texas, Porter & Savignano, 1990; Alabama, Landry, 2004) and *Linepithema humile* (Hawaii, Cole *et al.*, 1992; North Carolina, Human & Gordon, 1997) have reported significant suppression in Collembolan abundance in the presence of invasive ants. If Collembola have strong impacts on nutrient cycling in other systems as they appear to have in this and other systems (Anderson & Ineson, 1984; Ingham *et al.*, 1985; Mikola & Setala, 1998; Dunham, 2008), important indirect effects on ecosystem processes may be a widespread outcome in other ant-invaded systems as well and deserve further testing.

Wasmannia auropunctata is an invasive ant, thought to be on the verge of a pan-tropical expansion (Wetterer & Porter, 2003). Given the numerous negative impacts on native communities, biodiversity and ecosystem processes suggested by this study, invasive expansion of *W. auropunctata* should be closely monitored and steps should be taken to limit the expansion of this destructive species' range. The facilitation of spread by logging and oil industries in Gabon and other areas of Central Africa is apparent and critical steps are needed from

these companies to eliminate sources of invasion and to restrict further spread through careful management practices.

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