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Inferring ant (Hymenoptera: Formicidae) dietary responses to experimental drought in a tropical rainforest using stable isotopes

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Abstract

With higher frequency and intensity of droughts predicted for many tropical forests, understanding the responses of plants and animals to changed climatic conditions will be increasingly important. Ants are considered ecosystem engineers in tropical rainforests due to their abundance and the diversity of functional roles they perform. Function in animals is often dictated by diet, with diets of rainforest ants ranging from purely carnivorous to mostly plant based. Stable isotopes of nitrogen and carbon have been utilised to infer information on diets, and more broadly on changes in ecosystem dynamics and water-use in plants. We used stable isotopes to investigate the potential effects of drought on the diets of two dominant ant species (*Anonychomyrma gilberti* and *Oecophylla smaragdina*) in an in-situ through-fall experiment in lowland rainforest. We observed a significant and sustained increase of δ^{15}N in both ant species in the drought experiment and during the dry season in the control treatment. We also detected elevated although much smaller levels of δ^{15}N values in leaves of some drought-stressed plant species. There was no detectable difference of the experiment for ant or plant δ^{13}C values. We conclude that the observed elevation in ants' δ^{15}N in response to drought may be partly caused by a drought-induced shift in δ^{15}N in plants. Although, with average nitrogen enrichment values of 2.7‰ and 3.2‰, for *A. gilberti* and *O. smaragdina*, respectively, it is consistent with these ants deriving nitrogen largely from prey. Because ants are such dominant species in rainforests, even small trophic shifts in response to climate change if widespread could have broad implications for species interactions and food webs. Importantly, it is possible that such a change could potentially mediate the effects of drought on tropical forests if herbivory on drought-stressed plants is diminished by ant predation.

KEYWORDS
ants, drought experiment, rainforests, stable isotope analysis, trophic position

INTRODUCTION

There is a limited understanding of the effects of drought on tropical invertebrates. The few large-scale manipulative experiments that have been implemented in tropical forests have focused on tree physiological responses, stand dynamics and the broader implications for ecosystem function and global feedback loops (Apgaua et al. 2015; da Costa et al. 2010; Metcalfe et al. 2010; Rowland et al. 2015; Tng et al. 2018). Yet forest community composition is as much influenced by top-down pressures as bottom-up (Terborgh 1992); with interactions between insects, disease and drought, now commonly associated with global patterns in forest dieback (Anderegg et al. 2015; Weed et al. 2013). One early tropical example of this interaction is a drought study from Borneo where elevated seedling mortality was associated with an attack of pin hole borers during the 1998 El Niño drought (Bebber et al. 2002). Similarly, in the forest understorey of a drought experiment in Australian rainforest, we observed significant increases in termite activity, wood boring, disease and insect attack on drought-stressed plants (Gely et al. 2020; Tng et al. 2022). Although for canopy trees, this interaction may be less evident and harder to detect without specific focus. For example, a herbivory study based solely on litter fall collections in the drought experiment in eastern Amazon concluded there was no evidence of elevated leaf attack (Rowland et al. 2015). Whereas two canopy studies from the crane in Panama found elevated Lepidoptera outbreaks in rainforest *post* El Niño droughts in 1982–1983 (Aiello 1992) and 1997–1998 (Van Bael et al. 2004). Our recent review exploring how insect performance is affected by increasing drought severity predicted that most feeding guilds (except wood borers and bark beetles) would be negatively affected (Gely et al. 2020) which has been corroborated by a recent study that found approximately 3000 flying insect species were negatively influenced by drought induced-forest dieback and subsequent salvage logging (Sire et al. 2022) in southern France.

Drought could also have important indirect effects on species which if pervasive may result in cascading effects on food webs and altered community composition. For example, drought can alter invertebrate diets through a change in the quantity and quality of available food (Gely et al. 2020; Kume et al. 2007; Phillips et al. 2018; Walter et al. 2012) such as a decline in floral size, number of flowers and nectar volume (Kuppler & Kotowska 2021). Such changes in food availability may affect herbivores but also species at higher trophic levels, which are expected to be more vulnerable to climate change because they have to adjust not only to the direct physiological effects from changing climate but also to changes in species interactions from lower trophic levels (Voigt et al. 2003; Zhang et al. 2017). Consequently, assessing how drought affects food webs may be an important avenue to better understand its indirect effects on communities and ecosystem functions.

Stable isotope analysis has become a standard tool to examine food webs through evaluating if an animal's diet is mostly plant- or animal-based and subsequently determining its trophic position (Hyodo 2015; Potapov et al. 2019; Vander Zanden et al. 2015). One application is via the stable isotope measure delta N (δ^{15}N), which refers to the ratio of the heavier isotope, nitrogen-15 (^{15}N), to the lighter isotope, nitrogen-14 (^{14}N). When an organism consumes food, it tends to excrete the lighter isotope (^{14}N) and store the heavier isotope in tissues (Vander Zanden et al. 2015). In terrestrial ecosystems, plants set the baseline for δ^{15}N, with insect herbivores having similar or slightly higher values depending on if they feed on plant sap or leaves (Fagan et al. 2002). As insects consume other animals, their δ^{15}N values increase revealing a species' trophic position, which can change in response to altered environmental conditions or species interactions (Tillberg et al. 2007).

In this study, we use stable isotopes to examine how experimental drought has affected the trophic position of two dominant ant species *Oecophylla smaragdina* and *Anonychomyrma gilberti* in lowland rainforest. Prior to the installation of the drought experiment, stable isotopes of 50 ant species were studied in the same area, including *O. smaragdina* and *A. gilbeti* colonies (Blüthgen et al. 2003). In this earlier study, ant carbon and nitrogen isotope compositions were compared with ant-tended homopterans, 11 different arthropods (herbivores to predators) and leaves from 25 plant species visited by ants for floral and extra-floral nectar or honey-dew homopterans (Blüthgen et al. 2003). The results from this earlier study found that overall the isotope signatures of homopterans was within the same range as plants and did not differ significantly (plant mean ± *SD*, $\delta^{13}C = -28.5 \pm 1.7‰$, $\delta^{15}N = 2.2 \pm 1.3‰$), whereas ants differed by being more enriched by ~5‰ δ^{13}C and ~3‰ δ^{15}N for *O. smaragdina* and by ~4‰ δ^{13}C and ~2‰ δ^{15}N for *A. gilberti* (Blüthgen et al. 2003). The trophic position of these two species was considered intermediate compared with the other ant species, indicating an omnivorous diet based on predation, trophobioses and nectarivory. The authors concluded that trophic positions in ants may be better measured by variation in δ^{15}N rather than examining the variability of plant substrates (Blüthgen et al. 2003).

Here, we follow on from this earlier work and ask the following: (1) does experimental drought affect the diet of these two ant species (as determined by stable isotopes)? (2) Is there a relationship between soil moisture availability and stable isotope composition of the ant species? We hypothesise that ants may become more predatory in the drought experiment if nectar and honeydew resources are less abundant on drought-stressed plants, in which case δ^{15}N and δ^{13}C values are expected to be significantly more enriched in comparison with ants collected from non-treated sites. Furthermore, we hypothesise that a similar pattern may be evident in ants in the control treatment in response to seasonal changes in precipitation (measured via soil moisture).

MATERIALS AND METHODS

Study site

The study was located in a lowland tropical rainforest at the Daintree Rainforest Observatory, Cape Tribulation, north-eastern Australia (16°06′20″S 145°26′40″E, 50 m a.s.l). This site experiences a mean annual rainfall of

4900 mm $year^{-1}$ and a pronounced dry season from April to November and is located on rocky acidic, dystrophic soils, with tree canopy heights ranging from 24 to 47 m (Tng et al. 2016). Floristically, the forest is classified as complex mesophyll vine forest and the 1-ha forest plot where the drought experiment was established has 84 vascular tree species and >700 individual trees (≥10 cm diameter-at-breast-height) (Laidlaw et al. 2007).

The Daintree drought experiment is a through-fall experiment installed and maintained continuously since May 2015 (Laurance 2015). The experiment comprises one 0.6-ha control plot with no drought infrastructure and two drought plots of ~0.2 ha, all located next to each other. Through-fall water removal was achieved by installing clear plastic panels and guttering at 1–2 m above the forest floor. Slits were cut in the plastic panels to enable trees to penetrate through the roofing. Leaf litter accumulating on the plastic panels were relocated every few weeks to the forest floor to maintain biogeochemical inputs (Laurance 2015).

We examined the effects of the experiment on soil water availability through the establishment of time domain reflectometry probes in nine soil pits, placed at depths from 0.1 to 1.5 m in five control and four drought locations (S. Laurance & M. Liddell unpublished data).

Ant collection for stable isotope analysis

The two most common ant species *A. gilberti* and *O. smaragdina* were hand-collected from the forest understorey bi-monthly from May 2016 to May 2018. Groups of ants (considered colonies) were located on the ground, foliage and tree stems, avoiding edges of the drought plots. For every ant colony detected, at least 10 worker ants were collected to make one sample. Over the 2-year sample period, 46 colonies were collected for *O. smaragdina* and 38 for *A. gilberti*. All samples were frozen on their day of collection. The gaster of each ant was cut off at the petiole and removed from the sample so undigested food would not alter the measure of isotopic composition of ant tissues (Feldhaar et al. 2010).

Stable isotope analysis

Ant samples were dried at 60°C for at least 48 h before being weighed. Each sample was homogenised, placed in tin capsules and analysed on an automated IsoPrime isotope-ratio mass spectrometer. Stable isotope ratios are expressed in ‰ using the conventional delta (δ) notation as deviations from international standards (the IAEA international standard of atmospheric N_2 for nitrogen and the Vienna-Pee Dee Belemnite equivalent for carbon). The ratio of the heavier isotope to the lighter isotope ($^{13}C/^{12}C$ for carbon; $^{15}N/^{14}N$ for nitrogen) was estimated by the equation:

$$\delta X = \left[\frac{R_{sample}}{R_{standard}}\right] - 1 \times 1000$$

where X is the heavier isotope and R is the ratio of heavy to light isotope.

Precision of this mass spectrometer for invertebrate samples is 0.2‰, calculated as the 90% confidence interval of differences between paired values from duplicate samples.

We compiled plant isotope data from concurrent leaf studies undertaken at the drought experiment (N. Vogado and S. Laurance, unpublished data). Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) samples come from eight canopy tree species that have been part of long-term study on drought effects on rainforests (Apgaua et al. 2015; Apgaua et al. 2016; Tng et al. 2018; Vogado et al. 2020). These ecological dominant species contribute >40% of the stand biomass due to their large size and high relative abundance. A total of 150 sun-exposed leaves were collected from the canopy ($n = 82$ control; $n = 77$ drought) and dried at 60°C for 7 days and then homogenised. All elemental and isotopic analyses were conducted at the Advanced Analytical Centre, James Cook University in Cairns, Australia. Isotope composition for carbon and nitrogen were determined using an elemental analyser (ECS 4010 CHNSO Analyzer; Costech Analytical Technologies INC, Valencia, CA, USA) fitted with a Costech Zero Blank Autosampler coupled via a ConFloIV to a Thermo Scientific Delta V^{PLUS} using Continuous-Flow Isotope Ratio Mass Spectrometry (EA-IRMS). $\delta^{13}C$ and $\delta^{15}N$ values are reported as parts per million (‰) deviations from the VPDB and AIR reference scales, respectively.

Data analysis

We examined the effect of the drought experiment on $\delta^{15}N$ and $\delta^{13}C$ for the two ant species (*O. smaragdina* and *A. gilberti*) with Student's *t*-tests. The effects of drought on the stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) of canopy trees were determined with linear mixed effects models with variance estimated with restricted maximum likelihood (REML) using the nlme package (v3.1-163; Pinheiro et al. 2023). In the models, drought treatment was the fixed effect and variability among tree species captured as a random effect.

Furthermore, we assessed if drought affected seasonal patterns in the ant isotope signatures using generalised additive models (GAMs). GAMs assist in identifying seasonal patterns in biological and climatological responses using smoothed, nonlinear functions. These functions are more sensitive than simple wave functions, and they can take into account between-year seasonal variability (Wood 2017). We applied univariate GAMs with Gaussian family, attributing date of sampling as the predictor variable to characterise seasonal trends in isotope signatures and soil moisture data from the drought and control plots. The percent of deviance explained by fitted GAMs helped to determine if time of sampling was a good predictor variable for ant isotope signatures. Finally, we developed GAMs to explore the relationship between soil moisture and ant isotope signatures, using simple Spearman's rank correlation. All statistical analyses were performed in R version 3.5.1 (R Core Team 2013) with GAMs fitted using the mgcv package (Wood 2017).

RESULTS

We observed a significant effect of the drought experiment on mean nitrogen signatures in both ant species (Student's *t*-tests, *O. smaragdina*, $t = 7.147$, $p < 0.0001$, $df = 45$, Figure 1a; *A. gilberti*, $t = 8.613$, $p < 0.0001$, $df = 37$, Figure 1b). Ant colonies from the drought experiment were more enriched in ^{15}N increasing on average by +1.1‰ in *O. smaragdina* (mean ± *SD*, $\delta^{15}N_{control} = 5.5 \pm 0.7$‰ vs. $\delta^{15}N_{drought} = 6.6 \pm 0.5$‰) and +1.6‰ in *A. gilberti* ($\delta^{15}N_{control} = 4.6 \pm 0.5$‰ vs. $\delta^{15}N_{drought} = 6.2 \pm 0.5$‰). In contrast, no significant difference was found for $\delta^{13}C$ values between drought and control colonies (Student's *t*-tests, *O. smaragdina*, $t = 1.231$, $p = 0.225$, $df = 45$, Figure 1c; *A. gilberti*, $t = 0.511$, $p = 0.612$, $df = 37$, Figure 1d). Ant ^{13}C ranges were similar between colonies of the two species (*O. smaragdina* $\delta^{13}C_{control}$ −24.5‰ to −22.3‰; *A. gilberti* $\delta^{13}C_{control}$ − 25.2‰ to −23.1‰) and the drought and control treatments.

Similarly, we observed a significant effect of the drought experiment on $\delta^{15}N$ in leaf samples (Table 1, linear mixed effects model, $p < 0.001$, Figure 2a); however, no significant difference was found for leaf $\delta^{13}C$ between treatments (Table 1; Figure 2b). Leaf samples from the drought experiment were more enriched in $\delta^{15}N$,

TABLE 1 Linear mixed effects model (REML) results examining the effects of experimental drought on leaf stable isotopes ($\delta^{15}N$ and $\delta^{13}C$).

Variable	Fixed effect	*SD*	*df*	*t*-value	*p*-value
Leaf	Intercept	0.392	150	6.442	<0.0001
$\delta^{15}N$	Drought	0.161	150	3.909	<0.001
Leaf	Intercept	0.454	150	66.468	<0.0001
$\delta^{13}C$	Drought	0.191	150	0.306	0.759

Note: Models controlled for the random effects of tree species.

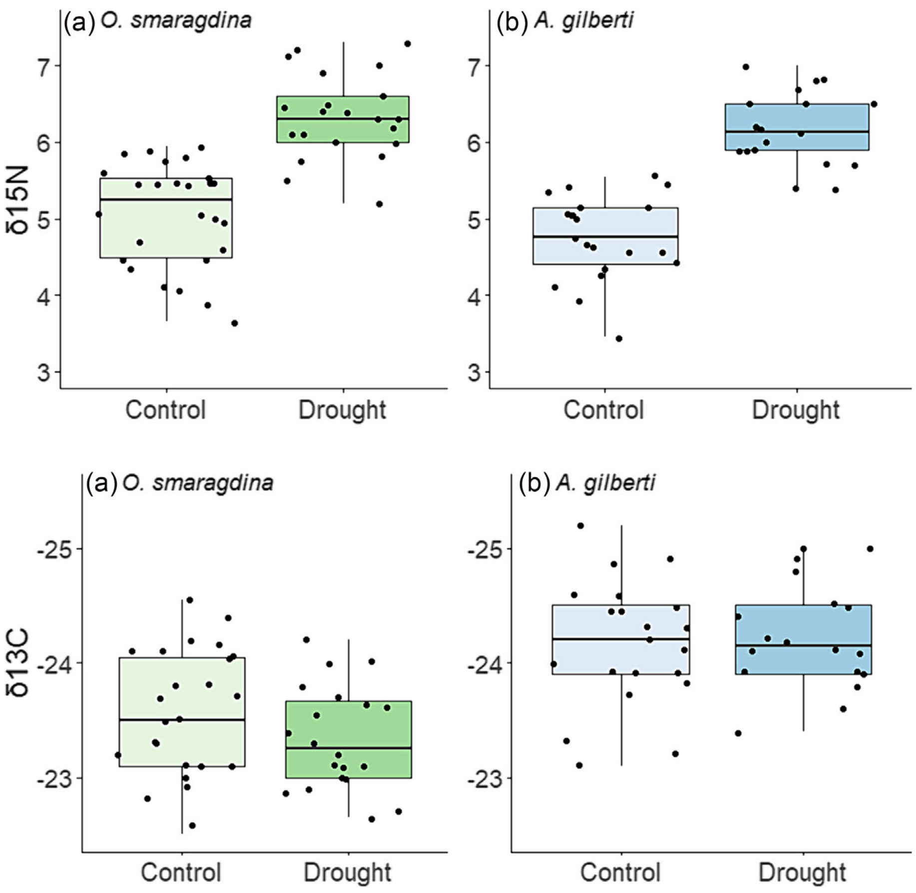

FIGURE 1 A comparison of stable isotope signatures of two dominant ant species between the Daintree drought experiment and adjacent control site for nitrogen ($\delta^{15}N$) (a) *Oecophylla smaragdina* ($n = 47$) and (b) *Anonychomyrma gilberti* ($n = 39$), and carbon ($\delta^{13}C$) (c) *O. smaragdina* ($n = 47$) and (d) *A. gilberti* ($n = 39$).

increasing on average by +0.6‰ (mean ± *SD*, $\delta^{15}N_{control} = 2.8 \pm 1.1$‰ vs. $\delta^{15}N_{drought} = 3.4 \pm 1.4$‰; Figure 2a), although it was highly variable between species. Leaf carbon isotopes showed a similar range between drought (mean ± *SD*, range, $\delta^{13}C_{control} = -30.1 \pm 1.4$‰, −27‰ to −32.9‰) and control samples ($\delta^{13}C_{drought} = -30 \pm 1.3$‰, −27.1‰ to −33.2‰) and also varied between species (Figure 2b).

The trophic enrichment of δ^{15}N of ants compared with leaf samples was higher for *O. smaragdina* (control +2.7‰ and drought +3.2‰) than for *A. gilberti* (control +1.8‰ and drought, +2.7‰), but δ^{13}C values were identical for both species and treatments (control ~6.7‰ and drought ~6.6‰). Both ant species also showed dry season enrichment of δ^{15}N in the colonies captured from the control but not the drought treatments. Through a comparison of ant isotope signatures and the available soil moisture over time, we determined with GAMs that time of year was a good predictor for δ^{15}N variations in the control treatment (Table 2). Seasonal peaks in ant δ^{15}N (Figure 3a) correlated significantly (Table 3, $P < 0.001$) with depressions in available soil moisture (Figure 3c) in the control whereas the drought experiment maintained consistently enriched levels of nitrogen in ants (Figure 3a) and low soil moisture throughout the study. There were no seasonal changes observed in ants' δ^{13}C in the control or drought treatments (Figure 3b).

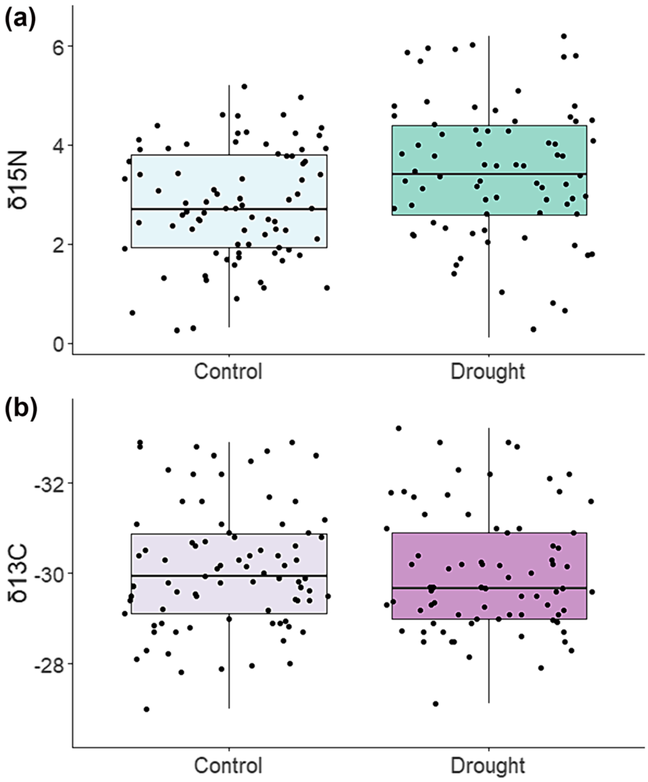

FIGURE 2 A comparison of isotope signatures for (a) δ^{15}N and (b) δ^{13}C of leaf samples ($n = 159$) collected from canopy tree species at the Daintree drought experiment and adjacent control treatment.

DISCUSSION

We found that experimental drought in a lowland tropical rainforest resulted in significant enrichment of δ^{15}N in the two dominant ant species (+1.1‰ in *O. smaragdina*, +1.6‰ in *A. gilberti*), but no change in δ^{13}C. This drought-induced elevation of ^{15}N was also observed in both ant species in the control treatment during the 2016 and 2017 dry seasons. Although we did not examine the isotope composition of dietary components directly, we compiled large concurrent isotope studies on rainforest trees where we also observed a significant but relatively small enrichment in leaf nitrogen in the drought (+0.6‰) but not in control treatments. Because leaves generally have higher nitrogen levels than sap, floral and extrafloral nectaries (because they are primary sites for photosynthesis and protein synthesis; Ueda et al. 2011), it is unlikely that the observed ^{15}N enrichment in the ant species is due solely to plant responses to drought. Further, previous studies from our study area have shown that the nitrogen content of nectar and honeydew is within the range of

TABLE 2 Summary of the seasonal patterns in soil moisture and ant isotope values of *Oecophylla smaragdina* and *Anonychomyrma gilberti* as expressed by the strength of the generalised additive models.

	Variable	*edf*	*F*	*p*	Explained deviance (%)
Control treatment	*O. smaragdina* δ^{15}N	6.998	11.00	<0.001	87.1
	δ^{13}C	1.408	0.19	0.770	4.9
	A. gilberti δ^{15}N	7.366	13.29	<0.001	90.3
	δ^{13}C	1.130	1.73	0.170	12.7
	Soil moisture	7.248	12.82	<0.010	96.8
Drought experiment	*O. smaragdina* δ^{15}N	1	0.01	0.920	0.1
	δ^{13}C	1	1.03	0.320	4.3
	A. gilberti δ^{15}N	1	0.10	0.760	0.6
	δ^{13}C	1.712	0.63	0.620	11.8
	Soil moisture	6.965	51.77	<0.001	99.1

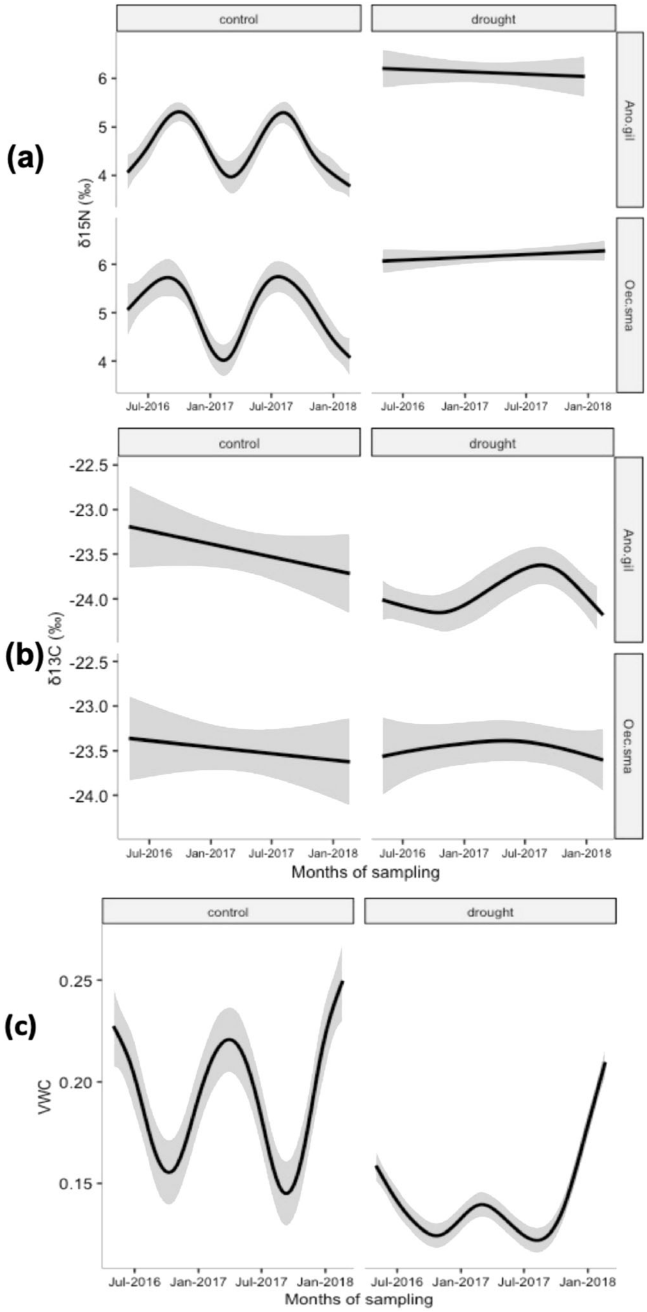

FIGURE 3 A time series of bimonthly isotopes samples for (a) δ^{15}N and (b) δ^{13}C for two rainforest ant species (*Oecophylla smaragdina* and *Anonychomyrma gilberti*) and (c) soil moisture (measured as volumetric water content [VWC]) at the control treatment and drought experiment. Solid lines are the generalised additive model predictions; grey areas are the 95% confidence intervals.

plants, and hence, they are unlikely to be significant source of nitrogen to ants (Blüthgen et al. 2003). We conclude, therefore, that relative to leaf δ^{15}N values, the trophic level enrichment of ^{15}N in the drought experiment of +2.7‰ and +3.2‰, for *A. gilberti* and *O. smaragdina*, respectively, is consistent with these ants increasing their dietary nitrogen from a higher proportion of prey items (Mooney & Tillberg 2005; Tillberg & Breed 2004).

Dietary flexibility has been previously recorded in *O. smaragdina* with colonies in young secondary forest found to be significantly more enriched in nitrogen than those in mature forests (Blüthgen et al. 2003). An increase in ant predation was hypothesised in secondary forests because of a lower availability of nectar and honeydew (Blüthgen et al. 2003). Similar trophic shifts have been observed in the invasive Argentine ant (*Linepithema humile*) in California, where ants at the invading edge were significantly enriched in δ^{15}N compared with the rear, due to ant diets being more prey-based at the front of the invasion (Tillberg et al. 2007). Although subsequent research has since found that post-invasion decline in trophic position in Argentine ants was not a general phenomenon, it is possible that flexibility in diet allowed for a trophic position that was consistent with dynamic ecological processes (Baratelli et al. 2023) or that diets change as resource availability changes. Likewise, the seasonal changes in ^{15}N we observed in our study species in the control sites are suggestive of dietary changes in response to resource availability.

Nectar and honeydew are principal components of *O. smaragdina* and *A. gilberti* diets (Blüthgen et al. 2004), and because it is widespread and generally abundant, it is considered the main reason for ant dominance in rainforest canopies (Davidson et al. 2003). We did not measure nectar and honeydew availability in this study. However, numerous studies from the same site indicate that the availability of plant-derived food such as flowering, extrafloral nectaries and leaf production, decline in the dry season when plants are water limited (Blüthgen et al. 2003; Boulter et al. 2006; Grimbacher & Stork 2009; Inkrot et al. 2007). Likewise, plants in the drought experiment have shown diminished productivity with reductions in fruiting activity, photosynthetic rate and stomatal conductance (Tng et al. 2022; Vogado et al. 2020). This decrease in plant productivity in response to water stress appears to have been mitigated in part in the drought experiment by an increase in rooting depth which we can infer from the observed increase in leaf δ^{15}N (Nel et al. 2018) and predicted from modelling (Pivovaroff et al. 2021). Leaf δ^{13}C can enrich in drought-stressed plants from increased stomatal closure (Farquhar et al. 1989) but was not found in this study. Hence, the dry-season and drought-induced changes in plant productivity observed to date have coincided with our detection of nitrogen enrichment in ants in the dry season and drought experiment. This finding provides indirect support for the hypothesis that sap-sucking insects may decline with increasing drought stress (Gely et al. 2020); henceforth, we believe that a fuller understanding would be achieved by direct observations of (1) different prey proportions being brought by ants to drought and control nests and (2) changes in ant-visitation and abundance of honeydew producing trophobionts.

Thus far, forest dieback studies have focussed on the direct effects of drought on plants and their interactions

TABLE 3 Strength and magnitude of the Spearman rank correlations between generalised additive model predictions of soil moisture and stable isotope values in *Oecophylla smaragdina* and *Anonychomyrma gilberti*.

	O. smaragdina		***A. gilberti***	
δ^{15}N				
Control treatment	$r_s = 0.366$	$p < 0.001$	$r_s = 0.684$	$p < 0.001$
Drought experiment	$r_s = 0.013$	0.417	$r_s = 0.053$	0.269
δ^{13}C				
Control treatment	$r_s = -0.0193$	0.763	$r_s = 0.0846$	0.395
Drought experiment	$r_s = 0.00127$	0.321	$r_s = 0.0197$	0.618

with herbivorous insects and disease (Anderegg et al. 2015; Desprez-Loustaua et al. 2006; Gely et al. 2020; Lantschner & Corley 2023). Indirect effects of climate change on species interactions such as predator–prey interactions are not well studied (Lord et al. 2017). Predators are known to have disproportional impacts on communities and ecosystems (Estes et al. 2011) and are predicted to be highly vulnerable to climate change because they not only need to respond to direct physiological changes of changing climate they are also reliant on changes throughout the food web (Voigt et al. 2003). Our finding that omnivorous ants may become more predatory in drought-affected forests has important implications for rainforest food webs and biodiversity and deserves further investigation into foraging behaviour. Given their large population size and territoriality (Dejean et al. 2007), ants could outcompete other invertebrate and vertebrate species and potentially excluding them from some areas. Additionally, some studies have found that drought leads to shrinking food webs and the loss of species from high trophic positions, such as birds (Campos-Cerqueira & Aide 2021; Roberts et al. 2019; Woodward et al. 2012). A positive outcome of canopy ants increasing their predatory behaviour would be a reduction in drought-induced herbivory in which case they could be potentially mediating the effects of climate change on plants.

Contextualising the effects of species interactions on ecosystem processes is inherently difficult because of the difference in spatial scales. Nonetheless, invertebrates potentially mediating the effects of drought on plants has been previously documented in other taxa such as dung beetles (Johnson et al., 2015) and termites (Ashton et al. 2019) and is a topic worthy for further research because species interactions are undoubtedly as important to ecosystem function as environmental conditions.

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CONFLICT OF INSTEREST STATEMENT

There were no conflicts of interest.

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REFERENCES

Aiello, A. (1992) Dry season strategies of two Panamanian butterflyspecies, *Anartia fatima* (Nymphalinae) and *Pierella luna luna* (Satyrinae) (Lepidoptera: Nymphalidae). In: Quintero, D. & Aiello, A. (Eds.) *Insects of Panama and Mesoamerica*. Oxford: Oxford University Press, pp. 573–575. https://doi.org/10.1093/oso/9780198540182.003.0037

Anderegg, W., Hicks, J., Fisher, R., et al. (2015) Tree mortality from drought, insects and their interactions in a changing climate. *New Phytologist*, 208(3), 674–683. Available from: https://doi.org/10.1111/nph.13477

Apgaua, D., Ishida, Y., Tng, D., et al. (2015) Functional traits and water transport strategies in lowland tropical rainforest trees. *PLoS ONE*, 10(6), e0130799. Available from: https://doi.org/10.1371/journal.pone.0130799

Apgaua, D., Tng, D., Cernusak, L., et al. (2016) Plant functional groups within a tropical forest exhibit different hydraulic strategies. *Functional Ecology*, 31, 582–591.

Ashton, L., Griffiths, H., Parr, C., et al. (2019) Termites mitigate the effects of drought in tropical rainforest. *Science*, 363(6423), 174–177. Available from: https://doi.org/10.1126/science.aau9565

Baratelli, E., Tillberg, C., Suarez, A., Menke, S., Naughton, I. & Holway, D. (2023) Variation in Argentine ant (*Linepithema humile*) trophic position as a function of time. *Biological Invasions*, 25(1), 133–140. Available from: https://doi.org/10.1007/s10530-022-02898-0

Bebber, D., Brown, N. & Speight, M. (2002) Drought and root herbivory in understorey *Parashorea* Kurz (Dipterocarpaceae) seedlings in Borneo. *Journal of Tropical Ecology*, 18(5), 795–804. Available from: https://doi.org/10.1017/S0266467402002511

Blüthgen, N., Gebauer, G. & Fiedler, K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia*, 137(3), 426–435. Available from: https://doi.org/10.1007/s00442-003-1347-8

Blüthgen, N., Stork, N.E. & Fiedler, K. (2004) Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos*, 106(2), 344–358. Available from: https://doi.org/10.1111/j.0030-1299.2004.12687.x

Boulter, S.L., Kitching, R.L., Zalucki, J.M. & Goodall, K.L. (2006) *Reproductive biology and pollination in rainforest trees: techniques for a community-level approach*. Cairns, Australia: Rainforest CRC.

Campos-Cerqueira, M. & Aide, T.M. (2021) Impacts of a drought and hurricane on tropical bird and frog distributions. *Ecosphere*, 12(1), e03352. Available from: https://doi.org/10.1002/ecs2.3352

da Costa, A.C.L., Galbraith, D., Almeida, S., Portela, B.T.T., da Costa, M., de Athaydes Silva Junior, J., et al. (2010) Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. *New Phytologist*, 187(3), 579–591. Available from: https://doi.org/10.1111/j.1469-8137.2010.03309.x

Davidson, D.W., Cook, S.C., Snelling, R.R. & Chua, T.H. (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. *Science*, 300(5621), 969–972. Available from: https://doi.org/10.1126/science.1082074

Dejean, A., Corbara, B., Orivel, J. & Leponce, M. (2007) Rainforest canopy ants: the implications of territoriality and predatory behavior. *Functional Ecosystems and Communities*, 1, 105–120.

Desprez-Loustaua, M., Marçaisb, B., Nageleisenc, L., Pioua, D. & Vannini, A. (2006) Interactive effects of drought and pathogens in forest trees. *Annals of Forest Science*, 63(6), 597–612. Available from: https://doi.org/10.1051/forest:2006040

Estes, J.A., Terborgh, J., Brashares, J., et al. (2011) Trophic downgrading of planet earth. *Science*, 15, 301–306.

Fagan, W.F., Siemann, E.C., Mitter, R.F., et al. (2002) Nitrogen in insects: implications for trophic complexity and species diversification. *American Naturalist*, 160(6), 784–802. Available from: https://doi.org/10.1086/343879

Farquhar, G., Ehleringer, J.R. & Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology*, 40(1), 503–537. Available from: https://doi.org/10.1146/annurev.pp.40.060189.002443

Feldhaar, H., Gebauer, G. & Blüthgen, N. (2010) Stable isotopes: past and future in exposing secrets of ant nutrition (Hymenoptera: Formicidae). *Myrmecological News*, 13, 13.

Gely, C., Laurance, S.G.W. & Stork, N.E. (2020) How do herbivorous insects respond to drought stress in trees? *Biological Reviews of the Cambridge Philosophical Society*, 95(2), 434–448. Available from: https://doi.org/10.1111/brv.12571

Grimbacher, P.S. & Stork, N.E. (2009) Seasonality of a diverse beetle assemblage inhabiting lowland tropical rain forest in Australia. *Biotropica*, 41(3), 328–337. Available from: https://doi.org/10.1111/j.1744-7429.2008.00477.x

Hyodo, F. (2015) Use of stable carbon and nitrogen isotopes in insect trophic ecology. *Entomological Science*, 18(3), 295–312. Available from: https://doi.org/10.1111/ens.12128

Inkrot, D., Satler, D., Geyer, C. & Morawetz, W. (2007) Flowering and fruiting phenology of *Normanbya normanbyi* (W. Hill) LH Bailey (*Arecaceae*), a palm endemic to the lowland tropical rainforest of north-eastern Australia. *Austral Ecology*, 32(1), 21–28. Available from: https://doi.org/10.1111/j.1442-9993.2007.01737.x

Johnson, S., Lopaticki, G., Barnett, K., Facey, S., Powell, J. & Hartley, S. (2015) An insect ecosystem engineer alleviates drought stress in plants without increasing plant susceptibility to an above-ground herbivore. *Functional Ecology*, 30, 894–902.

Kume, T., Takizawa, H., Yoshifuji, N., Tanaka, K., Tantasirin, C., Tanaka, N., et al. (2007) Impact of soil drought on sap flow and water status of evergreen trees in a tropical monsoon forest in northern Thailand. *Forest Ecology and Management*, 238(1-3), 220–230. Available from: https://doi.org/10.1016/j.foreco.2006.10.019

Kuppler, J. & Kotowska, M. (2021) A meta-analysis of responses in floral traits and flower–visitor interactions to water deficit. *Global Change Biology*, 27(13), 3095–3108. Available from: https://doi.org/10.1111/gcb.15621

Laidlaw, M., Kitching, R., Goodall, K., Small, A. & Stork, N. (2007) Temporal and spatial variation in an Australian tropical rainforest. *Austral Ecology*, 32(1), 10–20. Available from: https://doi.org/10.1111/j.1442-9993.2007.01739.x

Lantschner, M.V. & Corley, J.C. (2023) Spatiotemporal outbreak dynamics of bark and wood-boring insects. *Current Opinion in Insect Science*, 55, 101003. Available from: https://doi.org/10.1016/j.cois.2022.101003

Laurance, S.G.W. (2015) Rainforest in drought: creating a unique experiment to study the future of tropical forests. *Australasian Science Magazine*, 11.

Lord, J.P., Barry, J.P. & Graves, D. (2017) Impact of climate change on direct and indirect species interactions. *Marine Ecology Progress Series*, 571, 1–11. Available from: https://doi.org/10.3354/meps12148

Metcalfe, D.B., Meir, P., Aragão, L.E.O.C., Lobo-do-Vale, R., Galbraith, D., Fisher, R.A., et al. (2010) Shifts in plant respiration and carbon use efficiency at a large-scale drought experiment in the eastern Amazon. *New Phytologist*, 187(3), 608–621. Available from: https://doi.org/10.1111/j.1469-8137.2010.03319.x

Mooney, K.A. & Tillberg, C.V. (2005) Temporal and spatial variation to ant omnivory in pine forests. *Ecology*, 86(5), 1225–1235. Available from: https://doi.org/10.1890/04-0938

Nel, J.A., Craine, J.M. & Cramer, M.D. (2018) Correspondence between δ13C and δ15N in soils suggests coordinated fractionation processes for soil C and N. *Plant and Soil*, 423(1-2), 257–271. Available from: https://doi.org/10.1007/s11104-017-3500-x

Phillips, B., Shaw, R., Holland, M., et al. (2018) Drought reduces floral resources for pollinators. *Global Change Biology*, 24(7), 3226–3235. Available from: https://doi.org/10.1111/gcb.14130

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2023). *nlme: linear and nonlinear mixed effects models*. https://CRAN.R-project.org/package=nlme

Pivovaroff, A.L., McDowell, N.G., Rodrigues, T.B., et al. (2021) Stability of tropical forest tree carbon-water relations in a rainfall exclusion treatment through shifts in effective water uptake depth. *Global Change Biology*, 27(24), 6454–6466. Available from: https://doi.org/10.1111/gcb.15869

Potapov, A.M., Tiunov, A.V. & Scheu, S. (2019) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. *Biological Reviews*, 94(1), 37–59. Available from: https://doi.org/10.1111/brv.12434

Roberts, L.J., Burnett, R., Tietz, J. & Veloz, S. (2019) Recent drought and tree mortality effects on the avian community in southern Sierra Nevada: a glimpse of the future? *Ecological Applications*, 29(2), e01848. Available from: https://doi.org/10.1002/eap.1848

Rowland, L., da Costa, A.C.L., Galbraith, D.R., Oliveira, R.S., Binks, O.J., Oliveira, A.A.R., et al. (2015) Death from drought in tropical forest is triggered by hydraulics not carbon starvation. *Nature*, 528(7580), 119–123. Available from: https://doi.org/10.1038/nature15539

Sire, L., Yáñez, P.S., Wang, C., Bézier, A., Courtial, B., Cours, J., et al. (2022) Climate-induced forest dieback drives compositional changes in insect communities that are more pronounced for rare species. *Communications Biology*, 5(1), 57. Available from: https://doi.org/10.1038/s42003-021-02968-4

Terborgh, J. (1992) Maintenance of diversity in tropical forests. *Biotropica*, 24(2), 283–292. Available from: https://doi.org/10.2307/2388523

Tillberg, C.V. & Breed, M. (2004) Placing an omnivore in a complex food web: dietary contributions to adult biomass of an ant. *Biotropica*, 36, 266–271.

Tillberg, C.V., Holway, D.A., Lebrun, E. & Suarev, A.V. (2007) Trophic ecology of invasive Argentine ants in their native and introduced ranges. *PNAS*, 104(52), 20856–20861. Available from: https://doi.org/10.1073/pnas.0706903105

Tng, D., Apgaua, D., Campbell, M., Cox, C.J., Crayn, D.M., Ishida, F.Y., et al. (2016) Vegetation and floristics of a lowland tropical rainforest in northeast Australia. *Biodiversity Data Journal*, 4(4), e7599. Available from: https://doi.org/10.3897/BDJ.4.e7599

Tng, D.Y.P., Apgaua, D.M.G., Ishida, F.Y., et al. (2018) Rainforest trees respond to drought by modifying their hydraulic architecture. *Ecology and Evolution*, 8(24), 12479–12491. Available from: https://doi.org/10.1002/ece3.4601

Tng, D.Y.P., Apgaua, D.M.G., Paz, C.P., Dempsey, R.W., Cernusak, L.A., Liddell, M.J., et al. (2022) Drought reduces the growth and health of tropical rainforest understory plants. *Forest Ecology and Management*, 511, 120128. Available from: https://doi.org/10.1016/j.foreco.2022.120128

Ueda, M.E., Mizumachi, E. & Tokuchis, N. (2011) Foliar nitrogen turnover: differences among nitrogen absorbed at different times by *Quercus serrata* saplings. *Annals of Botany*, 108(1), 169–175. Available from: https://doi.org/10.1093/aob/mcr092

Van Bael, S., Aiello, A., Valderrama, A., Medianero, E., Samaniego, M. & Wright, S.J. (2004) General herbivore outbreak following an El Nino-related drought in alowland Panamanian forest. *Journal of Tropical Ecology*, 20(6), 625–633. Available from: https://doi.org/10.1017/S0266467404001725

Vander Zanden, M.J., Clayton, M.K., Moody, E.K., Solomon, C.T. & Weidel, B.C. (2015) Stable isotope turnover and half-life in animal tissues: a literature synthesis. *PLoS ONE*, 10(1), e0116182. Available from: https://doi.org/10.1371/journal.pone.0116182

Vogado, N.O., Liddell, M.J., Laurance, S.G.W., Campbell, M.J., Cheesman, A.W., Engert, J.E., et al. (2020) The effects of an experimental drought on the ecophysiology and fruiting phenology of a tropical rainforest palm. *Journal of Plant Ecology*, 13(6), 744–753. Available from: https://doi.org/10.1093/jpe/rtaa069

Voigt, W., Perner, J., Davis, A., et al. (2003) Trophic levels are differentially sensitive to climate. *Ecology*, 84(9), 2444–2453. Available from: https://doi.org/10.1890/02-0266

Walter, J., Hein, R., Auge, H., Beierkuhnlein, C., Löffler, S., Reifenrath, K., et al. (2012) How do extreme drought and plant community composition affect host plant metabolites and herbivore performance? *Arthropod-Plant Interactions*, 6(1), 15–25. Available from: https://doi.org/10.1007/s11829-011-9157-0

Weed, A., Ayres, M. & Hicke, J. (2013) Consequences of climate change for biotic disturbances in North American forests. *Ecological Monographs*, 83(4), 441–470. Available from: https://doi.org/10.1890/13-0160.1

Wood, S.N. (2017) *Generalized additive models: an introduction with R*. CRC: Chapman and Hall.

Woodward, G., Brown, L.E., Edwards, F.K., et al. (2012) Climate change impacts in multispecies systems: drought alters food web size structure in a field experiment. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 367, 2990–2997.

Zhang, L., Takahashi, D., Hartvig, M. & Andersen, K. (2017) Food-web dynamics under climate change. *Proceedings of the Royal Society*, 284, 20171772.

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