











# Nutrient use by tropical ant communities varies among three extensive elevational gradients: A cross-continental comparison

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## Abstract

**Aim:** Many studies demonstrate that climate limits invertebrates along tropical elevational gradients, but we have only a rudimentary understanding of the role of nutrient limitation and climatic seasonality. Here we examined the relationships between ant community structure, nutrient use and season along three undisturbed elevational gradients, each from a different continent.

**Location:** Ecuador (South America), Papua New Guinea (PNG: Oceania), Tanzania (Africa).

**Time period:** 2011–2014.

**Major taxa studied:** Ants.

**Methods:** Along each of the three gradients, we placed six distinct nutrient types (amino acid, sucrose, sucrose + amino acid, lipid, NaCl, H<sub>2</sub>O). In total, we distributed 2370 baits at 38 sites from 203m to 3972m. We used generalized linear models to test for the effects of elevation and season on ant species richness and activity and relative nutrient use. We also tested if changes in ant trophic guilds corresponded to changes in the use of particular nutrients.

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**Results:** Both species richness and activity decreased with elevation along each gradient. However, there were significant interaction effects among elevation, region and season, as ant activity in the dry season was higher in Ecuador and Tanzania but lower in PNG. Relative nutrient use varied among regions: ant preference for some nutrients changed with increasing elevation in Ecuador (decrease in lipid use) and Tanzania (decrease in amino acid and H<sub>2</sub>O use), while season affected nutrient use in PNG. There were common trends in trophic guilds along the three elevational gradients (e.g. proportional increase of predators), but these did not explain most of the nutrient use patterns.

**Main conclusion:** While the structure of ant communities changed similarly with elevation, both the seasonal and elevational effects on nutrient use by ants differed between continents. We argue that regional differences in climate and nutrient availability rather than ant functional composition shape nutrient use by ants.

#### KEYWORDS

elevational gradients, feeding preference, foraging, Formicidae, functional group, intercontinental differences, invertebrates, nutrient use, seasonal shifts, tropical forests

## 1 | INTRODUCTION

Understanding variations in community structure along environmental gradients is a central theme connecting biogeography and community ecology (Rahbek, 2005; Sanders & Rahbek, 2012). Because of the enormous biodiversity that rainforests harbour, tropical mountains are useful systems to study changes in community structure along climatic and resource-availability gradients (Peters et al., 2019; Rahbek et al., 2019). The most common two patterns for both species richness and abundance are a monotonic decrease and an unimodal relationship (a mid-peak) with increasing elevation (Colwell et al., 2016; McCain & Grytnes, 2010; Peters et al., 2016). In a similar manner to communities, nutrient limitation and use change along environmental and climatic gradients (Kaspari, 2020; Kaspari & Yanoviak, 2001; Remonti et al., 2011). Although our understanding of climatic and geometric constraints on animal communities has improved (e.g. Colwell et al., 2016), the influence of the environment on nutrient demands is rather poorly understood at the community level (Lasmar et al., 2023; Peters et al., 2014).

There is growing interest in how changes in community structure with elevation may influence the community function in mountain ecosystems, including resource use (Peters et al., 2019; Sundqvist et al., 2013). For instance, insights into how the relative abundance of trophic guilds changes with increasing elevation can help us assess the functional roles a taxon plays along mountain forest gradients (Mayr et al., 2020; Peters et al., 2014; Sam et al., 2017). Furthermore, even though tropical rainforests are relatively aseasonal, relatively small fluctuations in temperature and precipitation between the wet and dry seasons can significantly affect plant phenology and associated animal communities, and their resource use (Kishimoto-Yamada & Itioka, 2015;

Reich, 1995; Zimmerman et al., 2007). Such climatic impacts on tropical communities are expected to be greater for ectotherms like insects and in the regions where seasonality increases with elevation (Appelhans et al., 2016; Beck et al., 2008). However, the impacts of seasonality on patterns of diversity and resource use have typically not been studied in tropical mountains because most studies examine diversity only once during the peak activity of focal taxa (Beck et al., 2008; Lasmar et al., 2021; but see e.g. Bishop et al., 2014; Maicher et al., 2020; Tiede et al., 2017). Similarly, studies conducted across multiple geographic regions and different trophic guilds remain scarce, particularly for invertebrates (Beck et al., 2017). To fill these important knowledge gaps, we need to conduct standardized studies at the community level in different continents and seasons.

According to the optimal foraging theory, organisms should seek the resources and macronutrients they need most for their growth and metabolism (Stephens & Krebs, 2019). Therefore, communities are assumed to be limited by a certain set of nutrients and their availability. For example, sodium is one of the crucial elements for most animals and its availability varies across different environments (Kaspari, 2020). The availability of many other nutrients, particularly nitrogen and phosphorus, also limits geographically many taxa (Joern et al., 2012; Sundqvist et al., 2013). However, organisms from different trophic levels and functional groups are differentially constrained by particular macronutrients. Herbivores are more limited by sodium and proteins than are predators because predators can obtain these macronutrients in a higher accumulated concentration from their prey (Davidson, 1997; Kaspari, 2020). In contrast, predators are expected to be more limited by lipids and sugars (Kaspari & Yanoviak, 2001; Wilder et al., 2013). As nutrient cycling, plant productivity and the trophic structure of invertebrate communities vary along elevational and seasonal gradients (Hodkinson, 2005; Peters

et al., 2014; Reich, 1995), we assume that macronutrient constraints may also vary considerably along the same gradients.

Ants are ecologically important invertebrates that are sensitive to temperature at large and small spatiotemporal scales, making them a useful model taxon to study community variation with elevation and seasonality (Jacquemin et al., 2016; Jenkins et al., 2011; Sanders, 2002). They have a relatively complex nutritional ecology, as their larvae need lipids and proteins to grow while foraging adults (i.e. workers) are more dependent on carbohydrates, such as sugars from honeydew and plant nectar (Blüthgen & Fiedler, 2004; Csata & Dussutour, 2019). Furthermore, ants represent multiple trophic guilds, including specialized predators, generalized predators, scavengers, omnivores and herbivores (Folgarait, 1998; Lach et al., 2010). Many studies of ants and their nutritional ecology have been conducted under laboratory conditions, advancing our understanding of their behavioural and physiological requirements for key macronutrients (e.g. Cook et al., 2010; Dussutour & Simpson, 2009). However, only a handful of studies have been conducted in the field along broad geographic gradients at the level of entire communities and multiple macronutrients. From those studies, Peters et al. (2014) showed an increase in lipid use but a decrease in carbohydrates and sodium use with elevation in pristine habitats on Mt. Kilimanjaro. They speculated that preferences of particular nutrients by different ant trophic guilds, which also changed with elevation, may have caused these patterns. Recently, another study showed that ants at sites with higher annual seasonal temperature variation in Brazil preferred proportionally more carbohydrates and fewer other nutrients (Lasmar et al., 2021) and that arboreal ants prefer carbohydrates more than ground-dwelling predatory ants that visit more lipids in that region (Lasmar et al., 2023). Although none of these previous studies have directly examined how nutrient use varies among seasons or with elevation in more than a single mountain region, they allow us to make predictions about how the use of individual nutrients will change along broad climatic gradients.

Here we collated a large dataset from three separate studies on ants and their nutrient use, each conducted in different tropical region: Neotropics (Ecuador) by Tiede et al. (2017), Australasia (Papua New Guinea: PNG) by Moses (2015) and Afrotropics (Tanzania) by Peters et al. (2014). In all these studies the same baiting experiment with six different nutrients was used as in Peters et al. (2014). We focused our meta-analysis on the changes along three elevational gradients in three countries to disentangle for the first time the general effects of elevation and seasonality (i.e. dry versus wet season) on the ant community structure and their nutrient preferences. The three regions represent some of the last existing undisturbed continuous elevational gradients (i.e. the Eastern Cordillera, Mt. Wilhelm, Mt. Kilimanjaro) (Orivel et al., 2018; Peters et al., 2014; Tiede et al., 2017). For community structure, we focused at first on species richness, number of species occurrences and ant abundance as response variables to elevation and season. We predicted a linear decrease in the ant species richness and abundance with elevation at the three gradients

(Orivel et al., 2018; Peters et al., 2014; Tiede et al., 2017). Further, we ran a similar analysis on trophic guild level, where we predicted a decrease in abundance of ants dependent on plant-based resources (herbivorous and omnivorous species) but an increase in predators with increasing elevation (Peters et al., 2014). For nutrient use, we predicted that the use of lipids would increase with elevation and towards the wet (colder) seasons, as needs for thermal insulation and long-term food storage increase in colder environments and at higher trophic levels (Heinze et al., 2003; Wilder et al., 2013). Further, we predicted that carbohydrates, sodium and amino acids would be used more at lower elevations and in the drier (warmer) season to compensate for higher metabolic rates and colony growth at higher temperatures, in particular at lower trophic levels (Prather et al., 2018). We also predicted that the relative changes in ant trophic guild abundances would correlate with the individual nutrient use changes along elevational gradients (Peters et al., 2014). Finally, we expected the effects of seasonal variation on the ant communities and their nutrient use to be most pronounced in Tanzania, intermediate in Ecuador and least pronounced in Papua New Guinea, corresponding to the magnitude of temperature variation between the dry and wet seasons in each region.

## 2 | METHODS

### 2.1 | Novel meta-analysis and context to previously published studies

Studied elevational gradients represented three different zoogeographic regions in the equatorial tropics, each from a different continent and country: South America (Ecuador), Oceania (PNG) and Africa (Tanzania). In each of the regions, the sampling sites were distributed along an extensive elevational gradient (>2000m above sea level) (a.s.l.). We aimed to test the natural nutrient preferences of ants in relatively intact and comparable habitats, as was the focus of the study from PNG (Moses, 2015). Therefore, compared to the previous studies from Ecuador and Tanzania that sampled also disturbed sites (see Peters et al., 2014; Tiede et al., 2017), we focused in our joint analysis only on pristine vegetation habitats below the tree line in all regions. None of the previous analyses compared the different regions and their ant communities and nutrient use between seasons (Moses, 2015; Peters et al., 2014; Tiede et al., 2017). Furthermore, from the three studies, only Peters et al. (2014) have looked previously at variance in the ant species richness, trophic groups and relative nutrient use with elevation. In this study, we took the opportunity to conduct such a synthetic cross-continental study, following up on the analytical background introduced by Peters et al. (2014). Finally, as the data from Peters et al. (2014), Moses (2015) and Tiede et al. (2017) are not openly accessible, we deposited the collated dataset and all associated R-scripts for the sake of reproducibility (Mottl et al., 2023).

## 2.2 | Studied elevational gradients and their environmental characteristics

The Ecuadorian gradient was situated within the protected areas of Podocarpus National Park and Reserva Biológica San Francisco in the eastern Cordillera of the Andes in the provinces of Loja and Zamora-Chinchiipe, southern Ecuador (1000m–3000 m.a.s.l.) (Beck et al., 2008). The PNG elevational gradient was studied along Mt. Wilhelm, the highest peak in the Bismark range of the New Guinea island (4509 m.a.s.l.). The mountain gradient harbours high endemism of insects and plants and extends from the lowlands of Madang province (~200 m.a.s.l.) to the tree line (~3700 m.a.s.l.) (Leponce et al., 2016). The research sites along Mt. Wilhelm gradient were all located in pristine forests (for details about individual sites, see Leponce et al., 2016), whereas only undisturbed sites from Tiede et al. (2017) were considered. The African sites were situated along the slopes of Mt. Kilimanjaro, the highest African mountain (5895 m.a.s.l.). The natural vegetation types vary greatly along the mountain slopes, from open savannah habitat in the lowlands to closed-canopy diverse forests at mid-elevations, and *Podocarpus* and *Erica* forests at higher elevations. An increasing human impact during recent centuries has led to the conversion of many natural habitats in the lower part of the elevational gradient to agricultural lands (Peters et al., 2019). Here we focused only on the sites sampled in natural unconverted areas, from the lowland savannah (min. elevation 871 m) to the tree line (max. elevation 3849 m.a.s.l.) (Peters et al., 2014). For further environmental characteristics of the three study regions such as studied locations, elevational ranges and climatic variance see Table 1.

## 2.3 | Sampling design

Experimental nutrients were used in standardized amounts following Peters et al. (2014): (i) CHO (20% sucrose; 200g dissolved in 1000mL water), (ii) NaCl (1% salt; 10g dissolved in 1000mL water), (iii) amino acid (20%; 200g pure L-Glutamine powder dissolved in 1000mL water), (iv) CHO + amino acid (honeydew imitation; 500mL sucrose solution mixed with 500mL of the amino acid solution), (v) lipid (pure olive oil) and (vi) H<sub>2</sub>O (pure water). Each bait comprised a 50mL plastic tube filled at the bottom with 15 mL of the experimental solution, with a ball of cotton wool inserted and pressed onto the surface of the liquid. The ball was thus completely soaked when the tube was tilted horizontally, while the cotton wool prevented the solution from leaking out. The tubes were placed so that the rim was laid flat on the soil surface.

The same number of replicates and solutions per site were used in all three regions (six nutrient types and five replicates of each per site and per season: i.e. 30 baits for a sampling event). Each sampling event was either repeated in the dry and wet seasons (Ecuador, PNG), or they were divided by season according to the rainfall period when sampled (Tanzania). The spatial distribution of baits among the regions differed slightly (plots or transects), while the

TABLE 1 Environmental characteristics of the studied mountain regions.

Region (continent)	Gradient	Location	Studied range up to tree line (lowlands to highlands in m a.s.l.)	Climate	Dry season	Wet season	Precipitation ranges from lowlands to highlands	Annual temperature range from lowlands to highlands	Difference in mean daily temperatures between seasons in the mid-elevation
Ecuador (South America)	The eastern Cordillera of the Andes in the provinces of Loja and Zamora-Chinchiipe	Podocarpus National Park (4°17'0"S, 79°0'0"W) and Reserva Biológica San Francisco (3°58'30"S, 79°4'25"W)	Rainforests, 1050 to 2889	Humid	October–January (Rollenbeck & Bendix, 2011)	June–August (Rollenbeck & Bendix, 2011)	2000 to 4800 mm (Tiede et al., 2017).	20 to 9.5°C (Beck et al., 2008)	Dry season 3°C warmer (Beck et al., 2008)
Papua New Guinea (Oceania)	Bismark Range	Mt. Wilhelm (5°46'51.05"S, 145°1'50.39"E)	Rainforests, 203 to 3972 (Leponce et al., 2016)	Humid	June to September (Leponce et al., 2016; McAlpine et al., 1983)	Variable with peaks from December to January (Leponce et al., 2016; McAlpine et al., 1983)	3288 to 4400 mm (Sam et al., 2019)	27.4 to 8.4°C (Sam et al., 2019)	Similar temperatures throughout the year (Leponce et al., 2016)
Tanzania (Africa)	Single free-standing mountain	Mt. Kilimanjaro (2°45'–3°25'S, 37°00'–37°43'E)	Savannah to mountain forests, 881 to 3849 (Peters et al., 2014).	Seasonal	December to February, June to October (Appelhans et al., 2016)	March to May, and November (Appelhans et al., 2016)	850 to 3500 mm (Otte et al., 2017)	23 to 4°C (Appelhans et al., 2016)	dry season 5°C warmer (Appelhans et al., 2016)

minimum distance between the neighbouring baits was five metres in all regions. The exposure time of each bait was 1 h (PNG) or 2 h (Tanzania and Ecuador) and it was kept equal within each region, except in Ecuador, where it increased with elevation from 2 h in the lowlands by 1 h each 500 m a.s.l. (see Tiede et al., 2017). Despite the greater exposure times at higher elevations in Ecuador, the patterns in that region in terms of ant richness and occurrence were similar to those from the other regions (see Results), suggesting that the differences in exposure times do not strongly affect ecological patterns. A total of 2370 tubes were deployed globally, with 900 tubes exposed to ants at 15 sites in Ecuador between May and November 2014, and 960 tubes at 16 sites in PNG between May and November 2012 across the dry and wet seasons. In Tanzania, 510 tubes were exposed at 17 sites between March 2011 and October 2012 (seven sites sampled only in the dry season and 10 in the wet season). Changes in temperature and correlated shifts in vegetation structure and ecosystem functions are generally much stronger along the elevation gradient than among subsequent years. Therefore, we expect that the differences in the studied period have only a minor impact (in comparison to the climatic change with elevation) on patterns in community structure and nutrient use. For further details on sampling design and replication in each region see Supporting Information Appendix S1).

## 2.4 | Species identification and assignment to trophic guilds

Upon checking the baits, each tube was carefully closed, trapping the ants inside, taken to the laboratory and all ants were freeze-killed and transferred to vials with ethanol. All specimens collected in the three regions were then sorted, counted and identified to either species or morphospecies level, hereafter referred to as species. Only workers were observed inside the baits. We used Bolton (1994) and Shattuck (1999) for genera identification. For the species level, we consulted identifications with the reference collection at the Institute of Entomology, Biology Centre of the Czech Academy of Sciences and online databases ([www.antweb.org](http://www.antweb.org); [www.newguineants.org](http://www.newguineants.org)) for PNG and Tanzania, and Fernandez et al. (2019) for the Ecuador fauna. The species were then classified into three main functional groups, that is, generalists, herbivores/trophobionts and predators/scavengers (hereafter trophic guilds), following the guilds from Peters et al. (2014). The allocation of trophic guild to species was based on published data, our field observations and the measurements of the natural ratio of the stable  $\delta^{15}\text{N}$  isotopes for that species or, if not available, for the genus (see a list of the data sources in Appendix 1 and Supporting Information Appendix S2).

## 2.5 | Statistical analysis

Our interest was to test the hypotheses about the changes in ant community structure and relative nutrient use with elevation and

season at the community level, rather than at the level of individual baits, which are typically dominated by one or two ant species (see Results). In addition, the individual bait replicates within each sampled site and season (30 baits for the six nutrients, and five baits within one nutrient) were not spatially independent and all nested at the same elevation. Therefore, we followed the previous studies that used a similar experiment (Lasmar et al., 2021; Peters et al., 2014) and focused our analyses on each sampling event at a site as a replicate (i.e. community-level data for 30 baits pooled across the six nutrients or for five baits of each nutrient, depending on the model tested). This approach allowed us to compare the results directly to Peters et al. (2014). All analyses were conducted in R version 4.2.2 (R Core Team, 2022) using generalized linear models (GLMs), fitted by the 'glmmTMB' package. This package was selected due to its versatility of model fitting with options to account for various error distributions. We did not use mixed effects models (GLMMs) with the site as a random factor, because the sampling events were not replicated for all the factor levels and such models therefore suffered from false convergence or did not converge.

### 2.5.1 | Predictor selection

We used a unified statistical approach, in which a single 'final' most parsimonious model was selected from the model combinations of predictors tested (hereafter candidate models). The predictors were arranged in the candidate models in the order of their assumed importance for the invertebrate communities (i.e. elevation–region–season). The individual effects were tested in the candidate models, from the simplest model, in which each individual explanatory factor was initially included separately, to the complete model, which included all factors and their interactions. Both the first-order and higher order interaction were considered, where applicable. The candidate models were then ranked and their parsimony and explanatory power were compared using the corrected Akaike Information Criterion (AICc), selecting the model with the lowest AICc value (Burnham et al., 2011). In a few cases where multiple models yielded a similar AICc value ( $\Delta\text{AICc} < 2$ ), we preferred the model with higher explanatory power (with higher deviance-square:  $D^2$ ; note that 'glmmTMB' package uses absolute deviance, not relative, calculated as  $-2 * \text{likelihood ratio}$ ). For the elevational effect itself, we included both a linear effect (i.e. a first-order polynomial fit:  $\text{elevation}^1$ ) and a unimodal effect (i.e. a second-order polynomial fit:  $\text{elevation}^2$ ) in our candidate models so as to include also biologically relevant hump-shaped elevational patterns (Bishop et al., 2014). All candidate models considered are available online in Mottl et al. (2023). We used a conservative approach, where in addition to the selection of parsimony, we have applied a test of statistical significance using the likelihood ratio chi-square test, comparing model deviance with the null model (LR  $\chi^2$ ,  $p < 0.05$ ). The predictions of the final model were plotted using 'ggplot2' package (Wickham, 2016). In case of complex models (>two factors with interactions), the significances of individual

terms in each final model were assessed using Wald chi-square tests (Type = III) that are provided in Supporting Information.

Our analyses were divided into three groups. First, we examined changes in ant diversity and activity along the three gradients. This introductory analysis was performed to assess ant community structure for each sampled gradient and to test whether ants declined similarly with elevation in all three regions, since sites in each region were exposed to the bait for a different length of time. Second, we focused on the effects of nutrient type on bait occupancy and relative nutrient use following up on the previous studies (e.g. Peters et al., 2014). Finally, we tested, how the proportions of trophic guilds of ants changed with elevation and whether these groups were likely to cause (or contribute to) the observed relative changes in nutrient use.

### 2.5.2 | Ant diversity and activity along the elevational gradients

The following three response variables were tested: (i) species richness (number of ant species across all 30 baits), (ii) number of species occurrences (a measure of the ant species activity at the baits, calculated as the sum of the occurrences across all 30 baits of all species, with species represented across multiple baits being counted more than once) and (iii) ant abundance (total number of workers across all species and the 30 baits). A sampling unit was defined as a site sampled in a particular season ( $n = 79$ ). In the model candidates, we tested for the univariate effects of elevation (continuous predictor), region (three-level categorical factor), season (two-level binary factor) and their mutual interaction effects on the ants. All models were fitted using negative binomial error distribution.

### 2.5.3 | Changes in bait occupancies by ants

First, we assessed the bait occupancies by ants. As sites in Ecuador were not sampled above the limit of ant occurrence unlike in the other two regions, we excluded for that comparison all baits sampled above the elevations where no ants were observed in PNG and Tanzania (1980 baits and 66 sites retained to calculate the occupancies). For the sake of a visual comparison of overall bait occupancy in our experiment, we calculated the total proportion of baits occupied by ants for each of the six nutrients in each region across all baits and plotted (i) the average bait occupancy per region ( $\pm$ SD,  $n = 3$ ) and (ii) the overall occupancy between regions and the two seasons. Next, we calculated the proportions of baits occupied also for individual sampling events and each of the six nutrients (i.e. per site occupancy divided by nutrient type,  $n = 396$ ). We then tested the effects of nutrient type (six categories), elevation, region and season on the bait occupancies using beta-binomial error distributions and the GLM model framework as above, selecting one final model from the candidate models.

### 2.5.4 | Changes in relative nutrient use by ants in relation to region, elevation and season

Both bait occupancy per region and per site have shown a considerable variance among regions and the two seasons (including a significant interaction effect of nutrient type, region and season; see results). We therefore tested our hypotheses about the relative changes in nutrient use using separate analyses at the level of individual regions and nutrient types, rather than using a single model across all variables. This approach also allowed us to compare the results for each nutrient in each region with Peters et al. (2014), who used the same analytical framework. To reduce a bias in the estimates of relative nutrient preferences in these models due to the infrequent observations in high elevations, we did not include site replicates without ants and those with too low occupancy (i.e. less than three of 30 baits occupied). This further reduced our dataset to 23 of the 30 sites in Ecuador, 20 of the 32 sites in PNG and 10 of the 17 sites in Tanzania (total  $n = 53$ ). However, almost all ant occurrences (878 of 889) and species (182 of 183) were retained. Relative nutrient use was then calculated for each nutrient type and region, following Peters et al. (2014). We calculated the sum of species occurrences per nutrient type at each site and season as the sum of species counts across the five baits of that type at a given set of 30 baits. The relative use was defined as the ratio between the sum of species occurrences for each nutrient and the maximum number of species occurrences observed at that site in that season and region for any of the six nutrient types. By standardizing nutrient use in this way, we accounted for the considerable variation in ant abundance and/or activity among elevational sites (following Orivel et al., 2018; Peters et al., 2014). Thus, the highest value of relative nutrient use indicates the most preferred nutrient (one), while the lowest value (zero) indicates the least favoured nutrient (i.e. no ants recorded on it) at each elevational site. Note that the total value summed across the six nutrients is not equal to one, so more than one nutrient can have a maximum value, if it is equally preferred (different approach from a simple proportion, e.g. Lasmar et al., 2021). This is an important standardization for a meaningful interpretation of elevational (positive and negative) effects on relative nutrient use due to the natural strong decrease in ant activity with elevation (Supporting Information Figure S2).

To assess if the abundant species with large colonies (i.e. species visiting baits in high number of workers) disproportionately contribute to the relative nutrient changes, we also calculated the relative nutrient use using values based on the total ant abundances instead of species occurrences. To do this, the sums of worker abundances across the five baits for each site and nutrient were calculated. The values were transformed using  $\log(x + 1)$  before calculating nutrient use ratios. While the first occurrence-based approach focuses on activity-species richness patterns at baits and gives a higher weight to rare species, the abundance-based approach reflects the accumulation of ant numbers at the baits regardless of species richness and gives a higher weight to the abundant species.

The relative nutrient use of each of the six nutrient types was fitted for each continent separately using univariate effects of elevation (continuous predictor; using either first-order or second-order polynomial fit), season (two-level binary factor), their additive effect and their mutual interaction. The final model was chosen from the eight candidate models including null model, using the model ranking procedure explained above. The results were plotted for the significant ( $p < 0.05$ ), marginally non-significant ( $0.05 \leq p \leq 0.1$ ) and non-significant effects for each of the nutrients revealed by the LR  $\chi^2$  test (Supporting Information Tables S2 and S3). The models were run based on species occurrence or ant abundance.

### 2.5.5 | Variation in ant trophic guilds and their effect on relative nutrient use

For each site from the nutrient use analysis, we calculated the proportions of three trophic guilds as the number of ant species occurrences of each guild observed across all 30 baits divided by the total sum of ant species occurrences in those baits. This allowed us to assess changes in the trophic structure of ant communities visiting nutrient baits along elevational gradients and between seasons. Since the proportions of the three guilds are not independent (they sum to one for the three categories at each site), we did not consider the guild as a factor, but instead used a separate model for each guild as in Peters et al. (2014). We tested then the effects of elevation, region, season and their interactions, using a GLM framework of ranked model candidates as above, but with beta regression error distribution (Supporting Information Table S4). A first-order polynomial fit was used for the effect of elevation, as a second-order polynomial tended to overfit the proportional data.

Next, we used these proportions of trophic guilds as predictors of relative nutrient use. We wanted to determine whether communities with a high proportion of ants with a plant-based, animal-based, or omnivorous diet showed higher preferences for certain nutrients, which could explain some of the observed variance in nutrient use between gradients, as suggested by Peters et al. (2014). To test this hypothesis, we built a beta-binomial GLM with each trophic guild' proportion per site as a predictor and relative nutrient use as the dependent variable. Three models (i.e. three individual guild effects) were run for each combination of nutrient and region as model candidates and compared to the null model. Either the null model or a single guild effect (where significant), was used as the final model.

Finally, we wanted to test whether communities with a high proportion of ants with a plant-based, animal-based, or omnivorous diet showed higher preferences for certain nutrients after accounting for the changes in elevation and/or season. To do so, we have repeated the above analyses but used the 'final' model from the relative nutrient-use analyses as the 'null' model and tested, whether the addition of the information about trophic guild' proportion further improved that model.

## 3 | RESULTS

### 3.1 | Overview of ant communities

In the three regions, we sampled a total of 889 occurrences of 183 ant species across 2370 baits, of which 699 (30%) were occupied. The number of species per occupied bait varied from 1 to 4 (mean  $\pm$  SD =  $1.3 \pm 0.55$ ). The total number of species sampled in Ecuador was 62 from 900 baits (species occurrences (socs): 346, number of worker individuals (inds): 2613). In PNG, 79 species were found on 960 baits (socs: 372, inds: 4488) and 42 species were recorded on 510 baits in Tanzania (socs: 171, inds: 6446). The ant richness per occupied site (i.e. across 30 baits) varied from 1 to 19 (mean  $\pm$  SD =  $7.2 \pm 4.8$  species; Ecuador =  $7.1 \pm 4.9$ , PNG =  $8.2 \pm 5.1$ , Tanzania =  $5.6 \pm 3.7$ ) and the abundance from 1 to 3729 workers (mean  $\pm$  SD =  $226 \pm 523$  inds; Ecuador =  $97 \pm 119$ , PNG =  $204 \pm 254$ , Tanzania =  $586 \pm 1120$ ).

The assemblages at each region were completely distinct in species composition (no common species in two or more regions). Although the differences were high among the regions also at the genus level, there was some overlap. For example, of the five most abundant genera in terms of number of baits visited across the entire dataset, *Pheidole* and *Crematogaster* were present in all three regions. However, only *Pheidole* was among the five most common genera in all three regions when each region was considered separately (50% of species occurrences in Ecuador, 35% in PNG and 31% in Tanzania).

### 3.2 | Variation of ant diversity and activity with elevation and season

The highest elevation at which ants were observed was at 2889 m a.s.l. in Ecuador (i.e. the highest elevation sampled in that region), while ants did not occur above 2040 m in Tanzania and 2753 m a.s.l. in PNG. Ant species richness and number of species occurrences strongly varied with elevation and the effect was similar for all three regions (non-significant interaction of elevation and region for richness, and also for occurrence: Wald  $\chi^2 = 6.9$ ,  $p = 0.14$ ) (Table 2 and Supporting Information Table S1). Species richness in Ecuador, PNG and Tanzania decreased at a similar rate of about five species per 1000 m a.s.l. for the common elevational range sampled in all regions (i.e. from 1000 to 3000 m a.s.l.). However, the regions differed in species richness, with communities in Tanzania generally having on average fewer species (Figure 1a). Similar patterns were observed for the number of species occurrences, which decreased on average by about 20 occurrences each 1000 m a.s.l. in the season with the highest ant activity in all regions (Figure 1b). When considering the entire elevational ranges sampled, models predicted a small mid-elevational peak in PNG but a linear decline in species richness in the other two regions (Figure 1).

The elevation as an individual factor was a stronger predictor than the other variables combined (effect 'Elevation<sup>2</sup>': 80% of the

**TABLE 2** Generalized linear models (GLMs) testing the effects of environmental variables on ant species richness, activity (i.e. number of species occurrences and worker abundances across a set of 30 baits) and on bait occupancy (i.e. across a set of five baits per nutrient type) observed per site in the three regions Ecuador, Papua New Guinea and Tanzania. Individual effects of elevation (continuous predictor in m), region (three categories), season (binary factor: dry/wet) and their interactions were tested in a combination of candidate models from the simplest model with each of the individual explanatory factors present separately to the full model with all factors and all interactions. The models that performed best compared to the null models are shown in bold.

Dependent variable: data distribution	Model parameters							
Species richness: nbinom2 (link = "log")	AICc	$\Delta$ AICc	Residual df	Model df	Deviance	$D^2$	LR ( $X^2$ )	p-value
Null	444.4	144.1	77	2	440.3	0	NA	NA
<b>Model 1<sup>a</sup>: (elevation<sup>2</sup> + region) * season</b>	<b>301.2</b>	<b>0</b>	<b>68</b>	<b>11</b>	<b>275.2</b>	<b>0.375</b>	<b>165</b>	<b>***</b>
Species occurrence: nbinom2(link = "log")	AICc	$\Delta$ AICc	Residual fd	Model df	Deviance	$D^2$	LR ( $X^2$ )	p-value
Null	541.9	121.1	77	2	537.7	0	NA	NA
<b>Model 2: elevation<sup>2</sup> * region * season</b>	<b>420.7</b>	<b>0</b>	<b>60</b>	<b>19</b>	<b>369.9</b>	<b>0.312</b>	<b>167.9</b>	<b>***</b>
Worker abundance: nbinom2(link = "log")	AICc	$\Delta$ AICc	Residual fd	Model df	Deviance	$D^2$	LR ( $X^2$ )	p-value
Null	827	122.3	77	2	822.8	0	NA	NA
<b>Model 3: Elevation<sup>2</sup> * region</b>	<b>704.7</b>	<b>0</b>	<b>69</b>	<b>10</b>	<b>681.5</b>	<b>0.172</b>	<b>141.3</b>	<b>***</b>
Bait occupancy: betabinomial (link = "logit")	AICc	$\Delta$ AICc	Residual fd	Model df	Deviance	$D^2$	LR ( $X^2$ )	p-value
Null	1340.7	267.6	394	2	1336.7	0	NA	NA
<b>Model 4<sup>b</sup>: (nutrient type + elevation<sup>2</sup>) * region * season</b>	<b>1073.1</b>	<b>0</b>	<b>347</b>	<b>49</b>	<b>961</b>	<b>0.281</b>	<b>375.8</b>	<b>***</b>

Note: Only the name of the final best model compared to the null model from all candidate models is presented for the sake of brevity in the first column (i.e. Models 1 to 4 in bold with the lowest AICc and the highest  $D^2$ ). All candidate models compared are available online (Mottl et al., 2023). Significant p-values (<0.001) from the likelihood ratio test of deviance against the null model (LR  $X^2$ ) are marked with asterisks (\*\*\*). Significances of individual model terms are available in Supporting Information Table S1 and the fits of Models 1 to 4 are presented in Figures 1, S1 and S2, respectively. <sup>2</sup>A second-order polynomial function was used for the model fit; df, degrees of freedom;  $D^2$ , absolute deviance-square (pseudo- $R^2$ ); + additive effect; \* interaction effect including both first-order and higher order interactions.

<sup>a</sup>Model 1 formula: 'number of species ~ elevation + region + season + elevation:season + region:season'.

<sup>b</sup>Model 4 formula: 'occupancy ~ type + elevation + region + season + type:region + elevation:region + type:season + elevation:season + region:season + type:region:season + elevation:region:season'.

explained variance for richness, and 70% for occurrences, respectively, if compared to the final model). In both tested ant response variables, however, the region and season factors improve significantly the model fit in surplus the elevation (Table 2 and Supporting Information Table S1). There were significant interactions of region and season for both ant species richness and occurrence (Table 2 and Supporting Information Table S1ab). These differences were due to increased ant activity in the wet season compared to the dry season in PNG, while the opposite trend was true in the other regions (Figure 1). In contrast, the worker abundance did not change significantly with the season, but it declined more steeply with elevation in Tanzania, while it plateaued at low elevations in PNG and Ecuador (Table 2 and Supporting Information Figure S1 and Table S2c).

### 3.3 | Nutrient type effects on bait occupancy by ants

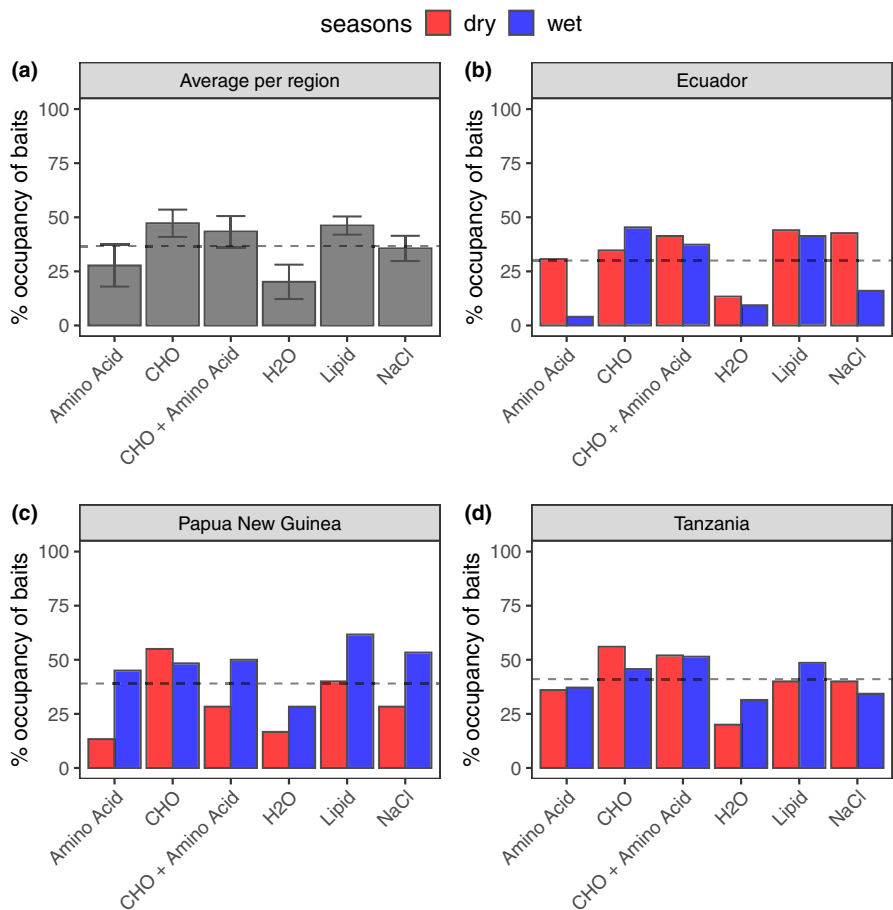
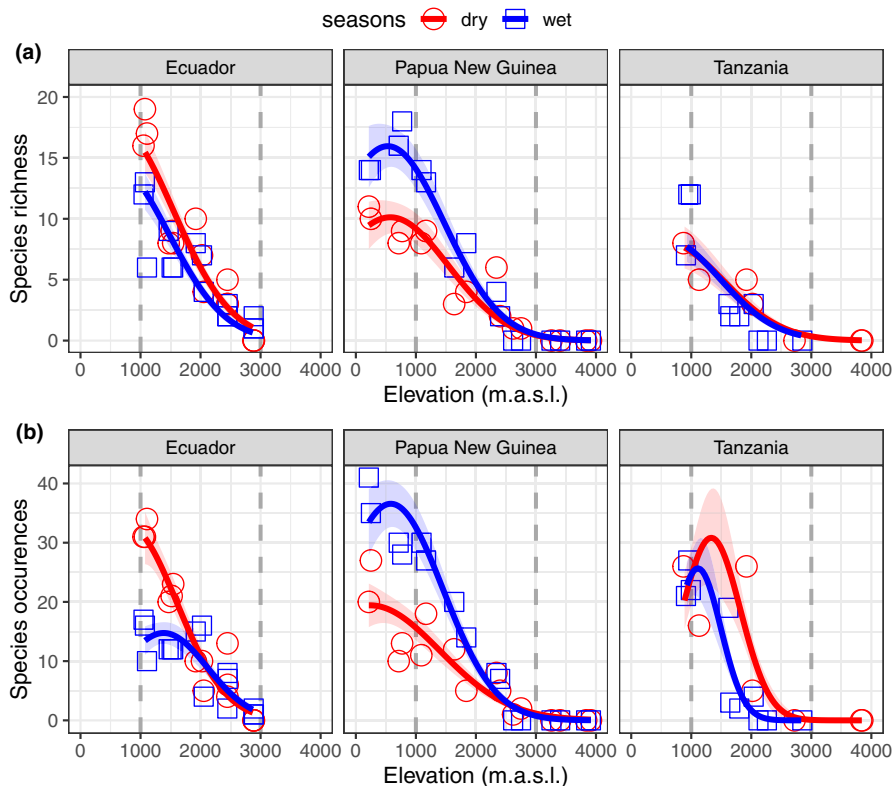
Of the six nutrients offered to the ants, CHO was most preferred by ants on average (47.2% bait occupancy), followed by lipid (46.2%), CHO+amino acid (43.4%) and NaCl (35.6%), while amino acid (27.7%) and H<sub>2</sub>O (20.2%) were the least preferred (Figure 2a).

The regional bait occupancy was relatively similar in Tanzania and PNG (39% and 41%, respectively), but was lower in Ecuador (30%) (Figure 2). The order of preferred nutrients varied among regions (Figure 2b–d). There were also seasonal differences: bait occupancy in PNG was higher during the wet than the dry season for most nutrients in contrast to the other two regions. In addition, both NaCl and amino acid bait occupancy were several times higher in the dry than in the wet season in Ecuador unlike in the other regions (Figure 2b). The expected higher limitation by H<sub>2</sub>O in Tanzania in the dry compared to the wet season was not reflected by the bait occupancy (20% vs. 32%) (Figure 2d). In the other two regions, the variance in occupancy of H<sub>2</sub>O mirrored the seasonal trends in other nutrients (Figure 2b,c).

Bait occupancy per site varied greatly across regions and with elevation for each nutrient, although including the interaction of elevation and nutrient type did not improve the fit, since visitation of all nutrients decreased relatively steeply towards the high elevations (the GLM final model: '(nutrient type + elevation<sup>2</sup>) \* region \* season') (Table 2 and Supporting Information Figure S2 and Table S1d). However, the interaction of nutrient type, region and season was highly significant (effect 'nutrient type: region: season', Wald  $X^2=30.8$ , df=10,  $p<0.001$ ).



**FIGURE 1** Variation in (a) ant species richness and (b) number of species occurrences with elevation in the three elevational gradients (Ecuador, Papua New Guinea and Tanzania regions). Each circle represents one site sampled in one season by 30 baits, with the species richness and number of species occurrences summed over these baits. Empty red circles indicate sites sampled in the dry season and empty blue squares those sampled in the wet season. Vertical dashed lines indicate the common range of elevations sampled. Red and blue lines show the trends for the sites sampled in dry and wet seasons, respectively. The transparent areas around curves indicate 95% confidence intervals from the GLMs. A second-order polynomial function is used for all model fits (see Table 2 and Supporting Information Table S1 for the model details).



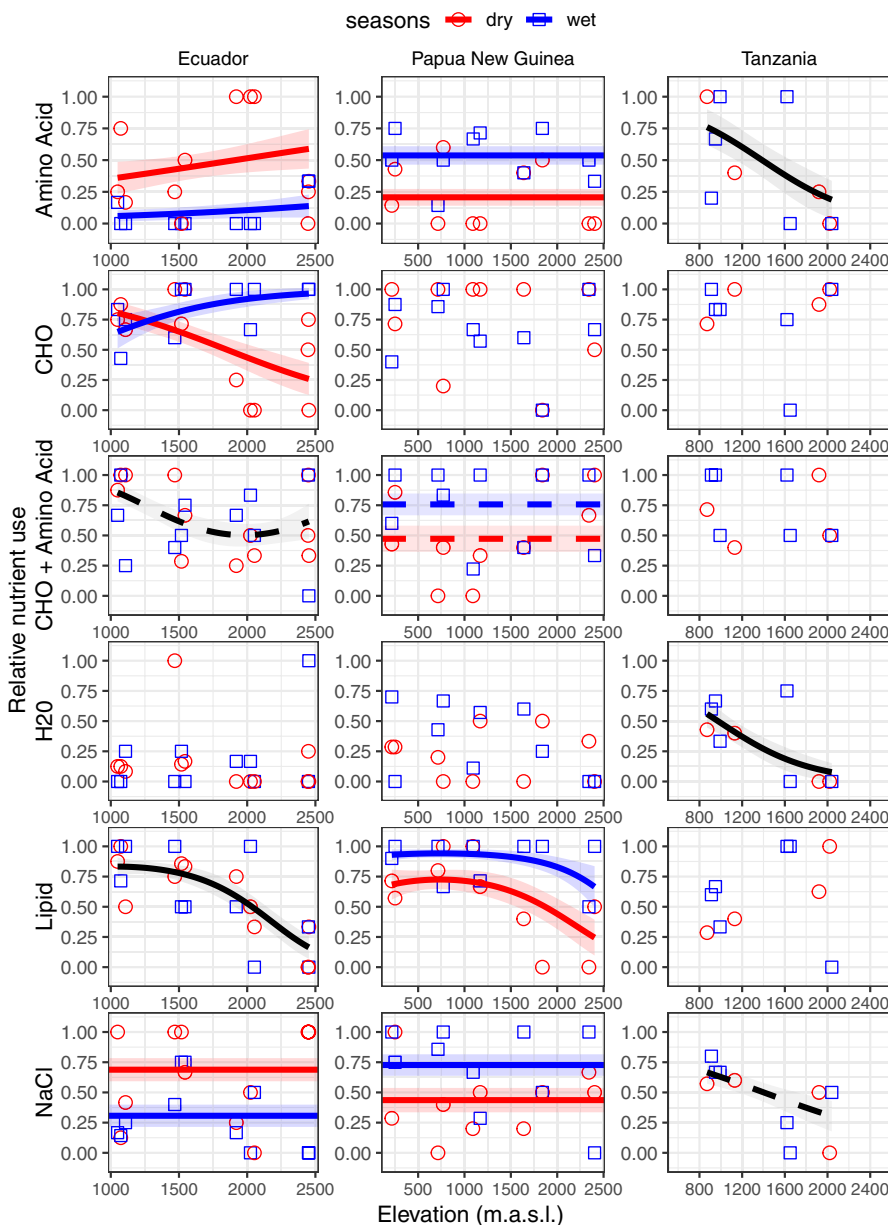
**FIGURE 2** The proportion of baits occupied (%) for each of the nutrient types in the three regions (Ecuador, Papua New Guinea, Tanzania): (a) mean occupancy per region ( $n=3$ ) with whiskers representing standard deviation, (b–d) occupancy by region and dry and wet seasons. Horizontal dash lines indicate the overall mean bait occupancy across all nutrients. The high elevations with ants not observed in PNG and Tanzania are excluded for comparability among the three regions (see Methods).

### 3.4 | Variation in the relative nutrient use by ants with elevation and season

There was a considerable lack of consistency in the use of individual nutrients along the elevational gradients. Relative nutrient use based on the number of species occurrences at baits varied among the regions, with relatively few changes with increasing elevation (Figure 3). The effects of elevation were relatively mild, with the strongest predicted change for the lipids in Ecuador and H<sub>2</sub>O in Tanzania ( $D^2 > 0.2$ ). Lipid use and CHO + amino acid use decreased, while amino acid use mildly increased along the elevational gradient in Ecuador. In contrast, there was only a slight decrease in lipid use in PNG (driven by the dry season). There were no changes with elevation in Tanzania in the use of lipids. Preference for amino acids, H<sub>2</sub>O

and NaCl decreased with elevation in Tanzania, but these effects were not replicated in the other two regions (Figure 3).

Regarding seasonal effects, ants used more amino acid and NaCl baits in the dry than in the wet season in Ecuador, while the opposite effects occurred in PNG for these two nutrients. There were no seasonal effects on nutrient use in Tanzania (Supporting Information Table S2). Interestingly, ants tended to utilize more CHO towards high elevations in the wet season in Ecuador, but there was a decrease in the same nutrient in the drier season at the same sites. In contrast, CHO use was random in the other two regions (Figure 3). Similar trends in relative nutrient use but less statistically significant were found when weighting the use by worker abundance rather than by species occurrence (Supporting Information Figure S3, Table S3).



**FIGURE 3** Variation in the relative nutrient use by ants with increasing elevation in each region (Ecuador, Papua New Guinea, Tanzania). The relative values for each of the six nutrients are based on the total number of species occurrences summed over the baits for the given nutrient, weighted by the most preferred nutrient at each site. The values range thus from 0 (no ants) to 1 (the most preferred nutrient) (see Methods). Sites with fewer than three visited baits of the 30 exposed are excluded. GLMs with beta-binomial error distributions are used to evaluate the effects of season, elevation and their interaction on the use of each nutrient (see Supporting Information Table S2 for model details). Solid lines indicate significant effects from the likelihood ratio test ( $p < 0.05$ ), while dashed lines indicate marginal effects ( $0.05 < p < 0.1$ ). Black lines denote the effects of elevation across both seasons when the season was not significant. Red and blue lines refer to the seasonal effects (dry and wet season, respectively) as follows: effect of season alone (horizontal straight lines for the mean); additive effect of season to elevation (parallel curves for each dry and wet season); the interaction effect of season and elevation (non-parallel red and blue curves). The transparent areas around each curve of corresponding colour indicate 95% confidence intervals from the GLMs.

### 3.5 | Variation in ant trophic guilds and their effect on nutrient use

The most common guild in all regions were omnivores, which accounted for >50% of the species occurrences at most sites, followed by predators (Figure 4). In contrast, the representation of herbivores was relatively low compared to the other two trophic groups, and they were in particular rare in PNG (only six occurrences of four herbivorous species). The final model used to predict the proportional changes of generalists and herbivores across sites was 'elevation<sup>1</sup> + region + season'. In contrast, season has no effect on the proportions of predators (the final model: 'elevation<sup>1</sup> \* region') (Supporting Information Table S4). In all regions, generalists and herbivores proportionally decreased on baits with increasing elevation, while the presence of predators increased from almost none in the lowest sites up to about half of the species occurrences at the highest sites (Figure 4). These trends in trophic groups with elevation and season were similar in all three regions, with the exception of a steeper increase in predators with elevation in Tanzania compared to the other two regions (Predators: effect 'elevation<sup>1</sup>: region', Wald  $\chi^2 = 12.2$ ,  $df = 2$ ,  $p = 0.002$ ) (Figure 4). Season effect did not change the slopes of any of the models, but the overall representation of generalists was higher in the wet than the dry season (Generalists: effect 'season', Wald  $\chi^2 = 4.8$ ,  $df = 1$ ,  $p = 0.03$ ), while the herbivores were more common in the dry season in all regions (Herbivores: effect 'season', Wald  $\chi^2 = 7.4$ ,  $df = 1$ ,  $p < 0.01$ ) (Figure 4).

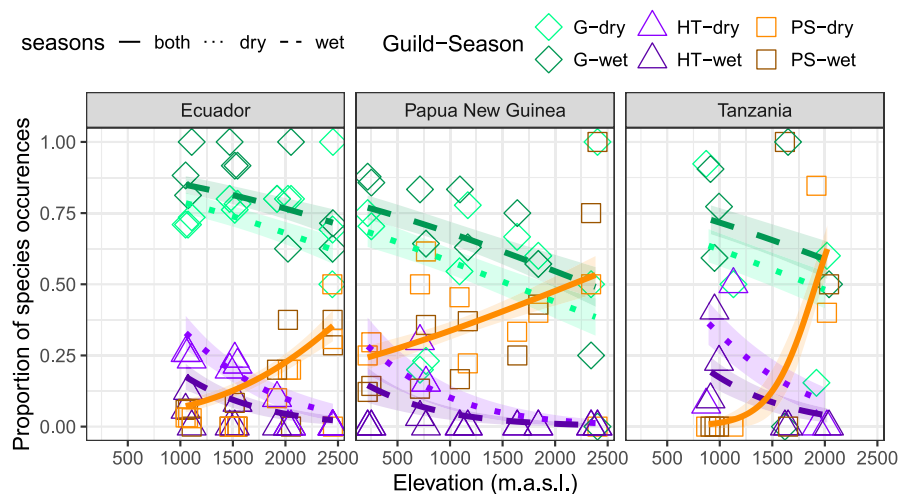
Despite this relatively high variation in trophic guilds with elevation, we did not find evidence that their proportional changes at our sites correlate strongly with observed patterns in nutrient use (Table 3). Most groups had no significant influence or were too rare

(e.g. herbivores in PNG) to drive any elevational and seasonal trends in the nutrient use (Figures 3, Table 3). An exception was the positive relationship between the proportion of generalists and NaCl preferences in PNG (LR test:  $p = 0.019$ ) and the relatively lower visitation of lipid baits and honeydew imitation baits at sites where predators and generalist were more common, respectively, in Ecuador (LR tests:  $p < 0.05$ ) (Figures 3 and 4, Table 3). However, even in those two cases  $D^2$  explained was low (explained variance <8%). No significant effects of any guild on nutrient use were found in Tanzania (Table 3).

Adding the effect of the trophic guild in surplus to the elevation and/or season did not significantly improve any of the nutrient use models. There was only marginal effect of generalist relative occurrence on NaCl use in Ecuador and PNG in addition to season (LR  $\chi^2$  test comparing 'season \* G' model to 'season' model:  $p < 0.1$ ) (Supporting Information Figure S4, Table S5).

## 4 | DISCUSSION

To our knowledge, this is the first cross-continental comparison of invertebrate responses and their nutrient preferences along elevation gradients using a unified methodology (Peters et al., 2014). Contrary to our predictions, for ants, one of the most abundant and ecologically significant insect groups, we did not find a common, cross-continental trend in the seasonal use of the six nutrients along elevational gradients. Furthermore, relative nutrient use did not change with elevation for most types of nutrients and zoogeographic regions we studied. This was an unexpected result, as we found similar declines in ant richness and abundance and relatively similar changes in the trophic structure of ant communities with



**FIGURE 4** Proportion of the different ant functional groups (trophic guilds) and their relationship with elevation in each region (Ecuador, Papua New Guinea, Tanzania). A first-order polynomial function is used to fit the elevation as a predictor for each trophic guild in a separate GLM model in combination with the effects of region and season (see the results and Supporting Information Table S4 for the details on the model used). The points represent the ratio between the sum of species occurrences for each guild and the total sum of species occurrences across all baits exposed at each site. Sites with fewer than three baits visited of the 30 exposed are excluded (see Methods). Green lines indicate relationships with elevation for generalists (G), violet lines for herbivores/trophobionts (HT) and orange lines for predators/scavengers (PS). Note the significant effect of season for G and HT guilds but PS (dry season: dotted line, wet season: dashed line). The transparent areas around each curve of the corresponding colour indicate 95% confidence intervals from the GLMs.

TABLE 3 Summary of GLM models testing for the effects of the proportional occurrence of ant trophic guilds (Figure 4) on the relative nutrient use of each of the six nutrient types in each of the three regions (Figure 3).

Nutrient	Region	Model	Intercept (coefficient)	Trophic guild (coefficient)	AICc	$\Delta$ AICc	Residual df	Model df	Deviance	D <sup>2</sup>	LR (X <sup>2</sup> )	p-value (* < 0.05)
Amino acid	Ecuador	G	0.92	-2.34	75.16	1.86	20	3	67.89	0.01	0.8	0.369
Amino acid	PNG	HT	-0.39	-5.12	71.75	1.31	17	3	64.25	0.02	1.5	0.223
Amino acid	Tanzania	Null	0.05	NA	38.05	0.00	8	2	32.33	0.00	NA	NA
CHO	Ecuador	Null	0.79	NA	77.39	0.00	21	2	72.79	0.00	NA	NA
CHO	PNG	Null	0.91	NA	68.08	0.00	18	2	63.38	0.00	NA	NA
CHO	Tanzania	Null	1.58	NA	28.41	0.00	8	2	22.70	0.00	NA	NA
<b>CHO + Amino acid</b>	<b>Ecuador</b>	<b>G</b>	<b>4.33</b>	<b>-4.53</b>	<b>77.98</b>	<b>1.46</b>	<b>20</b>	<b>3</b>	<b>70.72</b>	<b>0.05</b>	<b>3.9</b>	<b>0.049*</b>
<b>CHO + Amino acid</b>	<b>PNG</b>	<b>HT</b>	<b>0.72</b>	<b>-9.63</b>	<b>72.46</b>	<b>0.00</b>	<b>17</b>	<b>3</b>	<b>64.96</b>	<b>0.06</b>	<b>3.9</b>	<b>0.048*</b>
CHO + Amino acid	Tanzania	Null	1.10	NA	33.60	0.00	8	2	27.89	0.00	NA	NA
H <sub>2</sub> O	Ecuador	G	0.10	-2.21	63.26	1.96	20	3	56.00	0.01	0.7	0.401
H <sub>2</sub> O	PNG	G	-2.17	1.93	66.42	0.46	17	3	58.92	0.04	2.3	0.126
H <sub>2</sub> O	Tanzania	Null	-0.62	NA	35.79	0.00	8	2	30.08	0.00	NA	NA
<b>Lipid</b>	<b>Ecuador</b>	<b>PS</b>	<b>1.06</b>	<b>-5.06</b>	<b>78.79</b>	<b>0.00</b>	<b>20</b>	<b>3</b>	<b>71.53</b>	<b>0.07</b>	<b>5.5</b>	<b>0.019*</b>
Lipid	PNG	Null	1.12	NA	65.06	0.00	18	2	60.35	0.00	NA	NA
Lipid	Tanzania	PS	-0.10	1.25	37.93	1.84	7	3	27.93	0.08	2.4	0.118
NaCl	Ecuador	HT	-0.43	4.99	83.47	0.07	20	3	76.21	0.03	2.6	0.108
<b>NaCl</b>	<b>PNG</b>	<b>G</b>	<b>-1.65</b>	<b>3.13</b>	<b>72.27</b>	<b>0.00</b>	<b>17</b>	<b>3</b>	<b>64.77</b>	<b>0.08</b>	<b>5.5</b>	<b>0.019*</b>
NaCl	Tanzania	Null	0.13	NA	32.26	0.00	8	2	26.54	0.00	NA	NA

Note: A beta-binomial error distribution with a logit link function was used for the models. Only the model with the lowest AICc from the three trophic groups tested is considered (i.e. the strongest effect of a single guild). If the model performed worse than the null model ( $\Delta$ AICc > 2), only the latter is shown. Models with significant p-values ( $p < 0.05$ ) from the likelihood ratio test (LR with chi-square values) are marked by asterisk and in bold. NA is denoted, where the coefficient is not available and the null model performs better.

Abbreviations: D<sup>2</sup>, absolute deviance-square (pseudo-R<sup>2</sup>); Df, degrees of freedom; G, Generalists; HT, Herbivores/trophobionts; PS, Predators/scavengers.

elevation in all three regions. We expected that this consistency in trends in community structure would lead to common trends in nutrient use by ants (Davidson, 1997; Peters et al., 2014), but this was not the case. Our results suggest that differences in food availability, together with climatic variance between seasons, are responsible for the observed nutrient patterns in PNG and Ecuador, while in Tanzania, the elevational gradient, but not seasonality, plays some role.

We observed, however, some consistency in nutrient use among the regions if the elevational and seasonal variation is ignored. Visitation rates of CHO, lipids and CHO + amino acids baits were relatively high (over 40% of baits visited in all regions), while amino acids and NaCl were used less (<40%) and H<sub>2</sub>O the least from all nutrients (<20% of baits visited at most sites). A similarly high demand for energy-rich resources (carbohydrates and lipids) but rather lower preferences for salt, amino acid and H<sub>2</sub>O baits have been observed in other studies in both temperate and tropical regions (Fowler et al., 2014; Lasmar et al., 2021, 2023). The overall high preferences for these energy-rich resources point to high limitations of ant communities by carbohydrates and lipids, which agrees with studies stressing their importance for both ant workers and brood (Csata & Dussutour, 2019; Dussutour & Simpson, 2009).

#### 4.1 | Elevational variation in ant activity and its interaction with season

Our models suggested a nearly linear decrease in ant species richness, occurrences and worker abundance towards higher elevations. A relatively strong decrease was also observed for the bait occupancy. This was what we predicted, as bait visitations are influenced by ant activity, which as ectotherms are expected to decrease their foraging rates at lower temperatures (Orivel et al., 2018; Tiede et al., 2017). A small deviation from that decreasing pattern was found in the PNG gradient, where we observed a mid-elevation peak in ant richness and occurrence at ~800 m a.s.l. Such a peak was also revealed by the recent studies of the leaf litter and arboreal ant communities along the same gradient in PNG and might be explained by a more suitable range of biotic and abiotic conditions at that elevation (Moses et al., 2021; Plowman et al., 2020). We cannot exclude the existence of a similar pattern in the other regions, as our sampling in Ecuador and Tanzania took place above 800 m a.s.l. due to the restriction to pristine sites.

Despite these common trends with elevation, we found a significant interaction between the effects of elevation, season and region on the structure of ant communities in the species occurrence and bait occupancy models. We assume this effect was caused by the natural seasonal variation in ant activity between our geographic regions. Increased ant activity is expected in the dry season due to higher temperatures in Tanzania and Ecuador (Appelhans et al., 2016; Beck et al., 2008; Rollenbeck & Bendix, 2011). In contrast, PNG is part of Australasia and not far from

rainforests in North Australia that have colder dry seasons but warmer wet seasons, with a higher insect activity in the latter period (Frith & Frith, 1985; Wardhaugh et al., 2018). Nevertheless, the Mt. Wilhelm range experiences this climatic seasonal temperature variation most strongly above 2700 m a.s.l., where there are no ants, while the lower parts of the gradient are rather constant in temperatures (Leponce et al., 2016; Sam et al., 2019). We thus expected seasonal variance to have the highest effects on ant communities in Tanzania (the most seasonal region) but to have a negligible effect in PNG, but this was not the case. There are likely further drivers of these inter-continental differences in addition to climate. For example, varying food availability may explain some of the idiosyncratic patterns in ant activity across seasons found in our study (see below).

#### 4.2 | Elevational changes and relative nutrient use

We expected an increase in relative lipid use due to higher demand for fats in colder environments (Heinze et al., 2003) and a decrease in the use of other nutrients with elevation. However, our observations did not match this expectation. Furthermore, we could not replicate the results of an increased lipid use and a decreased carbohydrate use for the Mt. Kilimanjaro gradient (Peters et al., 2014). We suppose that the weak (marginally non-significant) effect of elevation on lipid use reported in this previous study and our focus on only the undisturbed pristine sites in our reanalysis might cause this discrepancy in Tanzania. Instead, relative lipid use did not change (Tanzania) or declined with elevation (Ecuador and PNG). The declining trend could be explained by a lower limitation of lipids in predators and omnivores at higher elevations in PNG and Ecuador, where these guilds were more abundant. Indeed, the proportion of predacious ants was negatively correlated with lipid use in Ecuador.

We expected that ant preference for amino acids and NaCl would increase at lower elevations because workers should have higher metabolic rates in hot environments (Prather et al., 2018). This factor may still have contributed to some of the trends in these two nutrients in Tanzania. However, given that H<sub>2</sub>O, NaCl and amino acid use did not change with elevation in PNG and Ecuador, it is more likely that the water deficit explains the variance in sodium and amino acid use in Tanzania. In fact, we observed a decrease in H<sub>2</sub>O use with increasing elevation attributable to the habitat change from drier savannahs towards wet rainforests along the Mt. Kilimanjaro gradient (Otte et al., 2017; Peters et al., 2019), while the changes in the relative occurrence of trophic guilds did not correlate with any of the nutrient changes. Overall, NaCl and amino acid appear to present a similar degree of limitation for ants along the parts of elevational gradients covered by rainforests. Alternatively, as the demand for sodium is also expected to increase in environments with heavier rainfall, that is, at higher elevations (Kaspari, 2020; Lasmar et al., 2021), the expected higher demand for sodium at higher temperatures in the lowlands could be mitigated.

The trade-off between compensating for higher metabolic rates in the warm lowlands and greater limitation by food availability in the cold highlands may explain the lack of strong elevational effects also for some other nutrients observed in most regions (e.g. CHO, CHO + amino acid). In PNG, in particular, we observed no changes with elevation except for a slight decrease in lipid use. The relatively consistent nutrient use at the community level in this region agrees with previous findings from preferential experiments conducted by placing different ant nutrient baits on understory plants, where no changes with elevation were found for amino acid or carbohydrate use (Orivel et al., 2018). The regional discrepancy might also be attributed to varying food availability along the three gradients: while potential prey densities increase along the Tanzania gradient (Supporting Information Figure S5), they do not change or rather decrease with elevation in PNG and Neotropical elevation gradients (Beck et al., 2008; Olson, 1994; Sam et al., 2020). Our results suggest that such nutrient limitations due to prey availability are mostly independent of the ant trophic guilds. This is perhaps not so surprising result, as most of the ant species we sampled are omnivorous, and even the predators and herbivorous ants are known to hunt or scavenge occasionally (Feldhaar et al., 2010; Lach et al., 2010). Interestingly, these results contrast with results of a recent study by Lasmar et al. (2023) that found a relatively strong effect of the ant trophic group on relative nutrient use for carbohydrates and lipids. The stronger explanatory power of the ant trophic guild effect in this study might have been due to sampling of the ants across different forest strata and exclusion of omnivores. In our study, the hypothesis of Peters et al. (2014) that different representation of trophic guilds drives changes in nutrient use along elevational gradients is not supported by our results for the ground-foraging assemblages. Instead, climatic variation and food availability in the mountains we studied are likely to be more important.

### 4.3 | Seasonal variation in relative nutrient use

Our results suggest that seasonal variation is an important factor that influences ant activity and relative nutrient use by ants in perhumid tropical forest gradients (i.e. PNG and Ecuador). A stronger effect of season and elevation on nutrient use was found when relative nutrient use was calculated from the number of species occurrences than from worker abundances across species. This has important implications because different ant species often perform different functional roles in ecosystems (Davidson, 1997; Folgarait, 1998). Indeed, previous modelling by Peters et al. (2014) showed that undisturbed communities with higher species richness use a greater diversity of nutrients than would be expected by chance.

The ants could thus play more functional roles during the period when they are more active and/or more restricted by certain nutrients and when the baits attract a higher diversity of species. This was the case in Ecuador (increased use of NaCl and amino acid in the dry season) and PNG (increased use of lipids, CHO and amino acids

in the wet season). We speculate that not only annual climatic variation but also differences in food availability between the dry and wet seasons between the Australasian and Neotropical regions may explain this difference. We sampled in the early wet season in PNG when there are fewer herbivorous insects than in the dry season (Sam et al., 2020; Whitfeld et al., 2012). In contrast to PNG, it is the dry season that is depleted of insect and plant resources in Neotropical forests (Fowler et al., 1993; Reich, 1995; Wolda, 1978). Consequently, Neotropical leaf-litter ants migrate into the soil in the dry season and are probably more limited by food compared to the wet season in this stratum than in PNG (Jacquemin et al., 2016; Neves et al., 2021; Tiede et al., 2017). We also observed an increasing use of CHO with elevation in the wet season but a decreasing trend for that nutrient in the dry season in Ecuador. This trend is probably related to the relatively cold microclimate in the high elevations of that mountain range (Beck et al., 2008), where the ants are more attracted to energy-rich resources in the wet compared to the dry season. For the dry season, we hypothesize that the limitation for carbohydrates might be higher for ants in the lowlands than highlands due to the increased activity of herbivorous ant species.

Surprisingly, we did not detect seasonal variation in the relative nutrient use by ants on the Mt. Kilimanjaro gradient, although the climatic variance is greatest in this region (Appelhans et al., 2016). We speculate that these relatively equal nutrient preferences across the two seasons might correspond to similar average prey availability across the year at the African sites (Supporting Information Figure S5).

### 4.4 | Limitations and methodological caveats

Our comparison of the three continents is a post hoc meta-analysis of three separate studies, and as such suffers from some variance in the spatio-temporal designs and site-replication in each region, a common problem when experiments are conducted separately. One of the concerns is the unequal exposure time of the baits, even though the standardized inert nutrients at the same concentration were used in all three regions. We do not feel this is an important bias for our conclusions, as the baits are visited by the ants quickly within the first hour in the tropics, and we found both abundant aggressive species and subdominant species at the sites, often sharing the same tubes, in all regions. Importantly, we observed a similar decline in species richness and occurrence towards the highest elevations in Ecuador as in the other two regions, and ants dropped to nearly no individuals at similar elevations in Ecuador as in PNG in rainforests (~3,000 m a.s.l.). Thus, the increasing exposure period of baits with elevation employed in the Ecuador gradient (Tiede et al., 2017) did not change the pattern of lower ant activity at higher elevations and it is unlikely to have affected the observed ant community structure and nutrient use patterns. Nevertheless, the lower replication of sites in Tanzania that were not repeatedly observed in dry and wet seasons could reduce the statistical interference of seasonal changes (increasing

the Type II error). More seasonal data and site replications from this region are needed (see also Peters et al., 2014 for discussion of the replication caveat).

Our analysis is a unique comparison that examines the invertebrate response to elevation and season across multiple tropical gradients. However, each gradient was sampled only within a period of 1 year. We still lack data from Equatorial regions that would replicate the same experiments not only across different mountains, but also across different years. This is an important step, as drought outbreaks like El Niño increase in intensity (Trenberth et al., 2014). In our study, we avoided such unusual weather periods and sampled a representative year. However, data from another study of ants from a South African seasonal region have shown that increased ant activity in one of the two seasons persists over several years despite some inter-annual variation along the elevational gradient (Bishop et al., 2014).

Studies like ours that use nutrient baits make the important assumption that resource visitation rates by ants reflect nutrient limitation in the environment. The alternative hypothesis that individual ant species specialize in certain resources, even when that nutrient is not limited in the environment could also play a role via evolutionary diet conservatism (Lasmar et al., 2023). This is particularly difficult to test along elevational tropical gradients, where each elevation band has a different species composition due to naturally high species turnover from low to high elevation (Colwell et al., 2016). To overcome this problem, we used the trophic guilds of ants as proxies to test, whether ecologically distinct species communities prefer different nutrients. Our analysis did not reveal any large effects on nutrient use by trophic guilds. However, we have to admit that there is still a lack of knowledge about the trophic ecology of most tropical species, as the trophic definition for many is a rough categorisation that reflects rather trophic information at the genus level. Future studies should look more deeply into the nutrient ecology of individual species or carry out the manipulative experiments. Recently, however, such manipulations have shown that ant communities altered their nutrient preferences in accordance with the nutrient limitation hypothesis (Ribeiro et al., 2019).

Finally, there is a lack of background data on food availability in the leaf litter from the three regions we studied, so the above information discussed is limited to vegetation (e.g. herbivorous insects and nectar). Yet measuring food limitations along wide environmental gradients in the tropical mountains remains a challenge even for vegetation. Future studies should also measure changes in food availability directly (see e.g. Camacho et al., 2022).

## 4.5 | Conclusions

We demonstrated common elevational changes across different tropical mountain regions in the species richness and individual abundance of an ecologically important ectotherm community, the ants, as well as similarities in the representation of their trophic guilds. However, the effects of seasonality differed among the three zoogeographic regions, which should be considered in future studies

of equatorial mountain regions. Our results do not support the hypothesis of relatively uniform nutrient use by ants along extensive elevational gradients in the tropics. Instead, there is a remarkable inconsistency in the relative use of different nutrients between regions at different elevations and seasons. These differences are likely due to regional differences in climatic conditions and nutrient availability, rather than the functional composition of the ants, which was similar in the three regions. Our study calls for a cautious interpretation of studies based on a single elevational gradient sampled during one period and it highlights the need to improve our background knowledge of nutrients and food availability on different continents.

## AUTHOR CONTRIBUTIONS

Jimmy Moses, Marcell K. Peters and Yvonne Tiede performed the experiments and led the data collection in the field, each in one of the three regions. Nathan J. Sanders designed the nutrient-baiting protocol. Jimmy Moses, Marcell K. Peters, Yvonne Tiede, Nina Farwig, Tom M. Fayle and Vojtech Novotny planned the field sampling. Jimmy Moses, Petr Klimes, Marcell K. Peters and David A. Donoso sorted and identified the ant taxa. Petr Klimes conceived the idea for the three-continental comparison. Jimmy Moses and Petr Klimes compiled the datasets and Jimmy Moses, Ondrej Mottl, Marcell K. Peters, and Petr Klimes conceived the analyses. Ondrej Mottl and Jimmy Moses wrote the code for the analyses, analysed the data and contributed to writing the methods and results. Petr Klimes wrote the draft and led the revision. All the authors contributed significantly to the revisions.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data, including the metadata and associated R scripts needed to reproduce all analyses and figures, are available in the Zenodo Digital Repository at <https://doi.org/10.5281/zenodo.8191946> (Mottl et al., 2023).

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## BIOSKETCH

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## APPENDIX 1

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