ORIGINAL ARTICLE

Variation in nutrient use in ant assemblages along an extensive elevational gradient on Mt Kilimanjaro

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ABSTRACT

Aim We used the extensive climatic and land-use gradient on Mt Kilimanjaro, Tanzania, to study large-scale variation of nutrient use in ground-foraging ant communities. In particular, we tested the hypothesis that recruitment of ants to six different nutrients would vary with elevation and between land-use regimes. We also tested whether the nutrient space (number and evenness of nutrients) used by ants decreases as species richness declines with elevation, a pattern expected because of complementarity in nutritional intake of species.

Location Mt Kilimanjaro, Tanzania.

Methods Standardized bait experiments with six nutrient treatments [carbohydrates (CHO), amino acid, CHO–amino acid mix, NaCl, H_2O and lipids] were performed at a total of 48 study sites in natural and managed ecosystems along an elevational gradient from 860 to 4390 m a.s.l. We used generalized linear models, information theory-based model inference and null model analyses to test hypotheses.

Results The species richness of ant communities declined with elevation in natural ecosystems but peaked at mid-elevations in managed ecosystems. We found that the use of four nutrients (NaCl, CHO, H_2O and lipids) varied with elevation and, in the case of NaCl and H_2O, with land use. Use of H_2O and CHO decreased with elevation, while lipid use increased. NaCl use increased with elevation in natural ecosystems but decreased in managed ecosystems. The nutrient space exploited by ant communities increased with ant species richness in natural ecosystems but decreased slightly in managed ecosystems. The difference could be because there are more trophic generalists in managed ecosystems and more specialists with complementary foraging niches in natural ecosystems.

Main conclusions Ant communities in different environments appear to be limited by different types and numbers of nutrients. This spatial heterogeneity in nutritional ecology is probably determined by both the environmental availability of nutrients and the functional composition of ant communities.

Keywords

Ants, elevational gradients, foraging preferences, habitat disturbance, land use, macronutrients, nutritional ecology, nutrient space, species richness, tropical mountain.

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INTRODUCTION

Animals require a suite of macro- and micronutrients in specific amounts and ratios to optimize their Darwinian fitness (Behmer, 2009; Raubenheimer *et al.*, 2009; Jensen *et al.*, 2012; Wilder *et al.*, 2013). However, nutrients are not evenly distributed, generating heterogeneity in the type and number of nutrients that limit communities in different environ-

ments (Krebs, 2009; Dudley *et al.*, 2012; Joern *et al.*, 2012). While a number of studies have investigated the nutritional ecology of individual species in particular environments or under laboratory conditions (Resasco *et al.*, 2014), much less is understood about how the nutrient demands of complex consumer communities change along large-scale environmental gradients (Kaspari *et al.*, 2008; Behmer, 2009).

Both nutrient availability in the environment and the trophic composition of communities are factors determining the nutritional demands and limitations of consumer taxa (Sterner & Elser, 2002). It has long been known that the availability of different macronutrients varies in space and can limit plant productivity in natural and agricultural ecosystems (von Liebig, 1855; Beer *et al.*, 2010; Mueller *et al.*, 2012). Similarly, for consumers, the reduced availability of specific nutrients generates nutritional imbalances that may ultimately limit the growth of individuals and populations and direct the foraging decisions of the animals towards the intake of nutrients in which they are deficient (Raubenheimer & Jones, 2006; Kaspari *et al.*, 2008, 2009; Behmer, 2009; Christensen *et al.*, 2010; Jensen *et al.*, 2012; Petry *et al.*, 2012).

Nutritional imbalances and limitations may also derive from the trophic ecology of species (Behmer & Joern, 2008; Kaspari *et al.*, 2008). For instance, because plants have 10–1000 times lower sodium and nitrogen concentrations than animal tissue, herbivores generally exhibit higher demands for sodium and proteins relative to predators and generalists (Strong *et al.*, 1984; Dudley *et al.*, 2012). Therefore, communities dominated by herbivores should show higher demands for sodium or proteins than communities dominated by predators or generalists (Kaspari *et al.*, 2008). Furthermore, because food webs become increasingly lipid-limited at higher trophic levels (Wilder *et al.*, 2013), demands for additional lipids may increase with increases in the relative abundance of higher trophic level consumers in communities.

Changes in the structure of ecological communities along environmental gradients may not only affect the type but also the different suites of nutrients that limit communities. Theoretically, the number and evenness (i.e. the relative abundance) of nutrients that are used by consumers (hereafter termed nutrient space) should increase with species richness, as the chances of there being species with complementary nutrient requirements in a community increases as the number of species increases (a concept derived from biodiversity–ecosystem function research; Tilman, 1999; Cardinale *et al.*, 2012). As variation in the diets of animals translates into variation in nutritional intake targets of foraging animals (Raubenheimer & Jones, 2006), communities with more species (and with complementary niches) may occupy a broader nutrient space than species-poor communities.

Human management practices alter the availability of nutrients in ecosystems considerably, but also the structure of consumer communities (Gibson *et al.*, 2011; Martin *et al.*, 2013). Many agricultural ecosystems are fertilized and, in dry areas, agricultural ecosystems are commonly irrigated to supply plants with limiting nutrients. These agricultural techniques to increase crop productivity probably also relax limitations of consumer communities for specific nutrients. In addition, human land use leads to changes in the relative abundance of trophic groups (Philpott *et al.*, 2010; Karp *et al.*, 2012), which may, as a consequence, affect nutritional demands for the entire community.

To date, most studies on the nutritional ecology of animals have been conducted in laboratories or, if they have been carried out in the field, at local scales (e.g. within one habitat), while only a few studies have assessed variation in the nutritional ecology of animal communities along extensive environmental gradients. Mountains can be test beds for studying gradients in the nutritional demands of animal communities at feasible spatial scales because climatic and biotic conditions change rapidly (and often systematically) with elevation (Körner, 2007), allowing macroecological studies at spatial scales of only a few kilometres (Sanders *et al.*, 2010; Sundqvist *et al.*, 2013).

In this study we used baiting experiments in natural and managed ecosystems on an extensive elevational gradient on Mt Kilimanjaro, Tanzania, to quantify whether demands of ant communities for six different nutrients [carbohydrates (CHO), lipids, NaCl, H_2O, amino acids and an CHO–amino acid mix] vary along environmental gradients. We also assessed patterns of species richness with elevation and tested whether a decline of ant diversity at higher elevations was related to reductions in the nutrient space used by ant communities.

MATERIALS AND METHODS

Study area and time of study

The study was conducted between February 2011 and November 2012 on the south-eastern slopes of Mt Kilimanjaro (Tanzania, East Africa; 2°45′–3°25′ S, 37°00′–37°43′ E). Mount Kilimanjaro has a north-west–south-east diameter of about 90 km and rises from the savanna plains at an elevation of 700 m a.s.l. to a snow-clad summit at 5895 m a.s.l. The region has a seasonal climate, with a long rainy season occurring from March to May and sporadic short rains occurring around November.

The mean annual temperature decreases linearly with elevation, with a lapse rate of *c.* 0.56 °C 100 m^{-1} starting at 23 °C in the foothills and decreasing to −7 °C at the top of the mountain (Hemp, 2006a). Annual precipitation exhibits a unimodal pattern, with *c.* 800–900 mm year^{-1} falling at the base of the mountain and *c.* 2700 mm year^{-1} falling at 2200 m a.s.l. (*c.* 2500–3000 mm), decreasing to < 500 mm year^{-1} in the afroalpine zone at the highest elevations. The natural vegetation at the base of the mountain is savanna (on the southern slopes of the mountain at *c.* 700–1100 m a.s.l.), shifting to submontane and lower montane forest (*c.* 1100–1800 m a.s.l.), *Ocotea* forest (*c.* 1800–2800 m a.s.l.),

Podocarpus forest (*c.* 2800–3200 m a.s.l.), *Erica* forest (*c.* 3200–4000 m a.s.l.) and alpine *Helichrysum* shrub vegetation (*c.* 4000–4600 m a.s.l.). As a result of the long history of human impact, the savanna and submontane forests have been largely cleared for agriculture. Maize plantations dominate areas that were formerly savanna vegetation, and agroforestry systems, coffee plantations and grasslands occur in areas that were formerly submontane forests. Ecosystems above 1800 m a.s.l. are protected as a national park (Kilimanjaro National Park) but are affected by selective logging and fire events, which have increased in frequency as a result of climatic changes during the last few decades (Hemp, 2005).

Study design and methodology

The study was carried out at a total of forty-eight 50 m × 50 m study sites, ranging in elevation from 860 to 4390 m a.s.l. (see Appendix S1 in Supporting Information). Twenty study sites were located in all the major natural ecosystems in the region (savanna, lower montane forest, *Ocotea*, *Podocarpus* and *Erica* forests and bushlands, and alpine *Helichrysum* vegetation), and the remaining 28 study sites were in agricultural ecosystems (maize plantations, agroforestry systems, coffee plantations and grasslands) or, at higher elevations, in ecosystems disturbed by human activities (logged *Ocotea* forest and fire-affected *Podocarpus* forest). The distances covered by the study sites of the same ecosystem type were in all cases at least 300 m. If possible, study sites were established in core zones of larger areas of the respective ecosystem type, so that effects of habitat ecotones were minimized.

The structure of ground-foraging ant communities and their nutritional demands were assessed by using bait experiments. Previous experiments have established that nutrient use patterns identified in bait experiments are indicative of nutritional limitations in ant communities (Kaspari *et al.*, 2008, 2009, 2012; Fowler *et al.*, 2014). Baits were 50-mL plastic Falcon® tubes (Becton Dickinson, Franklin Lakes, NJ) filled with 15 mL of one of the following six solutions: H_2O (tap water); NaCl (20 g cooking salt per 1 L H_2O); amino acid (200 g glutamine per 1 L H_2O); CHO (200 g sucrose per 1 L H_2O); CHO–amino acid mix (100 g sucrose + 100 g glutamine per 1 L H_2O); and lipids (pure olive oil). A cotton ball was added to the tubes to prevent the liquids leaking out of the tube. The cotton ball was pushed into the tube in such a way that it was soaked with the solution in the tube. At each of the 48 sites, we randomly placed five replicates of each bait type, i.e. a total of 30 baits per study site. The baits were placed on the ground at 5-m intervals along three parallel 50-m transects (distance between transects *c.* 15 m). Different types of nutrients were placed in a random order along the transects so that replicates of different nutrient types were interspersed with other nutrient types. Baits were placed at each site at times of high ant activity (i.e. in the early morning or late afternoon in the hot savanna, maize

fields and partly grassland sites; from late morning to early afternoon in forests and most other high elevational sites). After 2 h, we carefully collected the baits, ensuring that all ants at the baits were captured (see Appendix S2 for a detailed list of sampling times and the weather conditions at the times of sampling). The ants were sorted into morphotypes (see Appendix S3). One to three ants per morphotype were mounted and identified to genus level and subsequently to species or morphospecies level using Bolton (1994) and http://www.antweb.org/ (accessed 16 January 2013).

For each study site we counted the total number of species in all 30 bait tubes (a measure of species richness) and the number of species occurring at each of the 30 baits placed at each study site. Total species occurrence was calculated as the sum of the number of species found at each of the 30 baits per site. Species occurrence per bait type was calculated accordingly as the sum of species found at the five baits per site. For each of the six nutrient types, the relative nutrient use was defined as the ratio between species occurrence at the five baits of the respective nutrient type and the maximum number of species occurrences observed at any of the six nutrient types. By standardizing nutrient use in this way, we accounted for the considerable variation in ant abundance among sites, which would otherwise strongly bias nutrient-use patterns. Many studies (e.g. Kaspari *et al.*, 2008) on the foraging preferences of ants use sucrose as a reference nutrient because it is assumed that it is highly attractive to most ants. We did not do so, as the environmental gradient studied was very long and we observed that, in some habitats along the gradient, nutrients such as NaCl and lipids were more attractive to ants than sucrose. However, the patterns we documented were similar when sucrose was used as the reference nutrient. For each study site we defined the proportion of trophobionts, generalists and predators in the ant communities by dividing the number of species occurrences of the respective feeding group by the total number of species occurrences at all 30 baits per site. We allocated species to functional groups at the level of genera using a recent classification of East African ants based on stable isotope analyses, literature surveys and field observations (G. Fischer, F. Hita Garcia, J. W. Wägele, M. K. Peters, unpublished data; see Appendix S3 for details concerning the allocation of species to functional groups).

Statistical analysis

We used a stepwise approach to analyse the influence of elevation (a continuous variable) and land use (a two-level factor: natural or managed) on species richness and relative nutrient use. First, we checked for curvilinear relationships between elevation and the response variables by visually analysing the distribution of data points in scatter plots and performing generalized additive models using the *gam* function of the 'mgcv' package in R (R Core Team, 2013). Response variables were modelled as a smooth function of elevation. The *gam* function in this package by default automatically

estimates the degree of smoothing of the model terms by minimizing the generalized cross-validation criterion for the selection of smoothing parameters. Generalized additive models were performed for the two factor levels, natural and managed/disturbed ecosystems, separately.

Second, we used generalized linear models (GLMs) with a Gaussian (in the case of species richness) or binomial (in the case of relative nutrient use) data family to model the relationship between response variables and the explanatory variables elevation (ln-transformed) and land use and the interaction term land use × elevation. If we found curvilinear relationships in the first step we added the quadratic term elevation^2 and the corresponding interaction term habitat × elevation^2 to the GLMs. Levels of overdispersion were generally low, so statistical analyses of proportional data in the GLMs were run with the simple binomial rather than using the quasibinomial data family.

Finally, we constructed all the possible models based on this set of explanatory variables, including the null model (without any explanatory variables). For each response variable we selected a minimum adequate model using the Akaike information criterion (AIC) as the evaluation criterion. The AIC is based on information theory and evaluates models on the basis of model fit and model complexity (Burnham & Anderson, 2004). When comparing a series of models, the model with the lowest AIC value is considered to be the best. As our sample size was relatively low in comparison with the number of estimated parameters, we used the AIC with a second-order bias correction (AIC$_c$) instead of the standard AIC for model selection. We tested the significance of all the explanatory variables in the minimum adequate model based on classic null hypothesis testing. For all nutrients that were dissolved in water, we added the relative nutrient use of H_2O as an additional explanatory variable in the models and checked whether patterns between response and explanatory variables remained stable. We expected that, if the use of an H_2O-dissolved nutrient is because of an attraction to H_2O and not to the dissolved nutrient, the relationship between relative nutrient use and elevation and/or human land use would no longer be significant after controlling for relative H_2O use. We only report the results of this analysis if the results differed when H_2O was added as an explanatory variable in the model.

We tested whether the nutrient space used by ant communities (the diversity and evenness of nutrients exploited by ants) varied with elevation, land use and species richness by comparing the results of null models with patterns observed in ordinary linear models. The nutrient space used by ant communities was estimated for each study site by calculating the Shannon diversity index (which incorporates evenness) for the observed species occurrences at the six nutrient bait types. With six different types of nutrients, the Shannon diversity index may reach a maximum of 1.792 in the case of all six nutrients being visited by ants with an equal number of species occurrences per nutrient type, and a minimum of

0 if only one nutrient type is visited by ants, irrespective of the total number of species occurrences. As the probability of obtaining higher Shannon diversity indices increases with increasing ant abundance, the variation in the total number of species occurrences per study site has to be considered when analysing relationships between diversity in nutrient use and elevation, land use or species richness. We did this by comparing the measured nutrient-use diversity on sites with diversity estimates based on null models, i.e. with patterns expected when the occurrence of ants at the six different nutrient baits was determined by a random process. For each study site, we calculated 1000 null model estimates of the Shannon diversity index. The estimates for the null communities were based on a random allocation of the total number of species occurrences to the six different nutrient types. We plotted the distribution of null model estimates and measured the values of nutrient use diversity from scatter plots. For each study site we calculated the standardized difference between the mean null model estimate and the observed nutrient space as: Δnutrient space = (nutrient space observed − nutrient space predicted by null models)/SD null model predictions.

We analysed whether Δnutrient space is influenced by elevation, land use and species richness using ordinary linear models. If the nutrient space used by ant communities is a function of these variables, we expected differences to be non-randomly distributed along the range of values of explanatory variables. We note that, of course, the ants are not responding to elevation directly but rather to a suite of covarying biotic and abiotic factors (Sundqvist *et al.*, 2013). We simply used elevation in our analyses to encapsulate these variables.

RESULTS

The ant community at all baits comprised 79 species from 17 genera (see Appendix S3). In total, we found ants at 450 of the 1440 baits placed at 48 study sites along the Mt Kilimanjaro elevational gradient. Fifty-two of the 450 baits were occupied by two ant species and seven baits by three ant species, so the total number of species occurrences at baits ($n = 516$) was slightly higher than the number of baits occupied by ants. The minimum adequate model accounting for variation in species richness of ants at baits included elevation, the quadratic term elevation and the interaction term land use × elevation as explanatory variables (species richness AIC$_c$ = 217.2, $R^2 = 0.63$, $F_{5,42} = 14.61$, $P < 0.001$; P-values for all explanatory variables including quadratic terms and interaction terms were significant at a level of $P < 0.05$). The total number of ant species and species occurrences per site declined monotonically with increasing elevation in natural habitats, but peaked at mid-elevations in managed habitats (Fig. 1): the number of species was low at the base of the mountain, peaked at an elevation of *c.* 1350 m a.s.l. and declined at higher elevations. We did not detect any ants above 2270 m a.s.l.
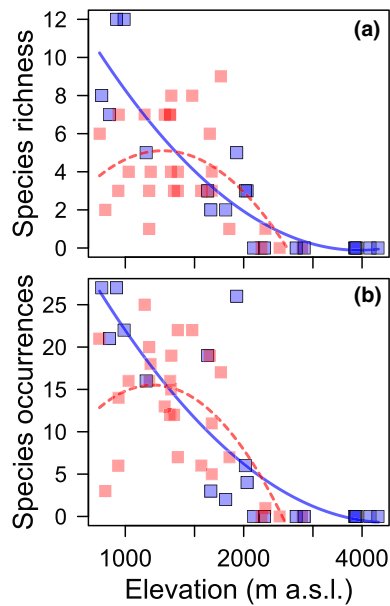
Figure 1 Ant (a) species richness and (b) species occurrences in relation to elevation at sites on Mt Kilimanjaro, Tanzania, in natural (black-rimmed blue squares, solid blue line; richness $r^2 = 0.82$, $P < 0.001$; occurrences $r^2 = 0.72$, $P < 0.001$) and managed/disturbed (red squares, dashed red line; richness $r^2 = 0.34$, $P < 0.01$; occurrences $r^2 = 0.46$, $P < 0.001$) habitats.

Figure 2 The proportion of different functional groups of ant species along the elevational gradient at sites on Mt Kilimanjaro, Tanzania, in (a) natural and (b) managed/disturbed habitats. Lines are model predictions based on generalized linear models (family = quasibinomial).

Forty-four of the 79 species found at baits belonged to generalist genera, 23 to predator/scavenger genera, and 12 to genera predominately feeding on honeydew or floral nectar (for simplicity hereafter simply called trophobiotic ants or trophobionts). The proportion of generalist ants was significantly higher in managed habitats (mean 80%) than in natural habitats (mean 57%) and decreased with elevation (GLM; elevation, $P < 0.01$; land use, $P < 0.01$; interaction, n.s.; Fig. 2). The proportion of trophobiotic ant species decreased with increasing elevation in natural habitats, while in managed habitats an inverse pattern was found (GLM; elevation, $P < 0.05$; land use, $P < 0.05$; interaction, $P < 0.05$). The proportion of predatory or scavenger species generally increased with elevation (GLM; elevation, $P < 0.01$; land use, n.s.; interaction, n.s.). For all three functional groups the same results were found when using absolute species occurrences instead of proportional data as the response variable.

Across all study sites, the ant communities most preferred CHO–amino acid baits (134 species occurrences) and CHO baits (133), followed by lipid baits (96), amino acid baits (62), NaCl baits (55) and H_2O (36). Minimum adequate models revealed that the relative use of nutrients varied strongly, as a function of both elevation and land use (Table 1, Fig. 3). The relative use of H_2O baits decreased with increasing elevation and was significantly higher in natural than in managed habitats. The relative use of NaCl baits showed contrasting patterns in natural and managed habitats. In natural habitats, the relative recruitment of ants at NaCl baits decreased along the elevational gradient, while
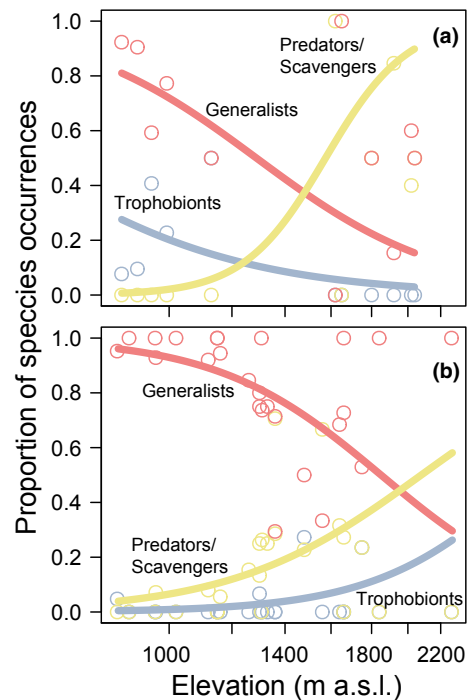
in managed habitats it increased with elevation. The relative use of CHO baits was highest at the base of the mountain and slightly decreased at elevations of *c.* 1300 m a.s.l., with no significant differences between natural and managed ecosystems. In contrast, the relative use of lipid baits slightly increased with increasing elevation in both natural and managed ecosystems. The minimum adequate model suggested that the relative use of amino acid baits decreased with increasing elevation and was significantly higher in natural than in managed habitats. However, after controlling for relative H_2O use, both effects (i.e. of elevation and land use) were no longer significant and the best supported model in terms of AIC_c was the null model. CHO–amino acid mix baits in managed systems had slightly higher ant recruitment than CHO–amino acid mix baits in natural ecosystems, although this effect was marginally insignificant ($P = 0.101$).

The relationship between elevation, species richness and the nutrient space used by ant communities differed between managed and natural ecosystems (Fig. 4). In natural ecosystems at low elevations, the nutrient space was broader than predicted by random processes of nutrient selection (in all five study sites at < 1300 m a.s.l. the observed nutrient use diversity was higher than the median null model estimates), while at elevations > 1300 m a.s.l. at five of six sites the nutrient space was more narrow. In managed ecosystems the nutrient space did not change along the elevational gradient.

Table 1 Minimum adequate models for six types of nutrient baits used to analyse ant recruitment along an elevational gradient on Mt Kilimanjaro, Tanzania, based on corrected Akaike information criterion (AIC_c) model selection. The values are parameter estimates derived from generalized linear models using a log link function.

Nutrient	Intercept	ln elevation	Land use	ln elevation × land use	AIC_c
H_2O	16.66	−2.45**	−0.85*		93.25
NaCl	14.08	−1.98*	−25.29*	3.40*	110.80
Carbohydrates (CHO)	16.06	−2.04*			89.72
Lipids	−8.00	1.16(*)			134.7
Amino acid†	−0.53				132.9
CHO–amino acid mix	0.97		0.66		105.9

†Final model after controlling for relative H_2O use.
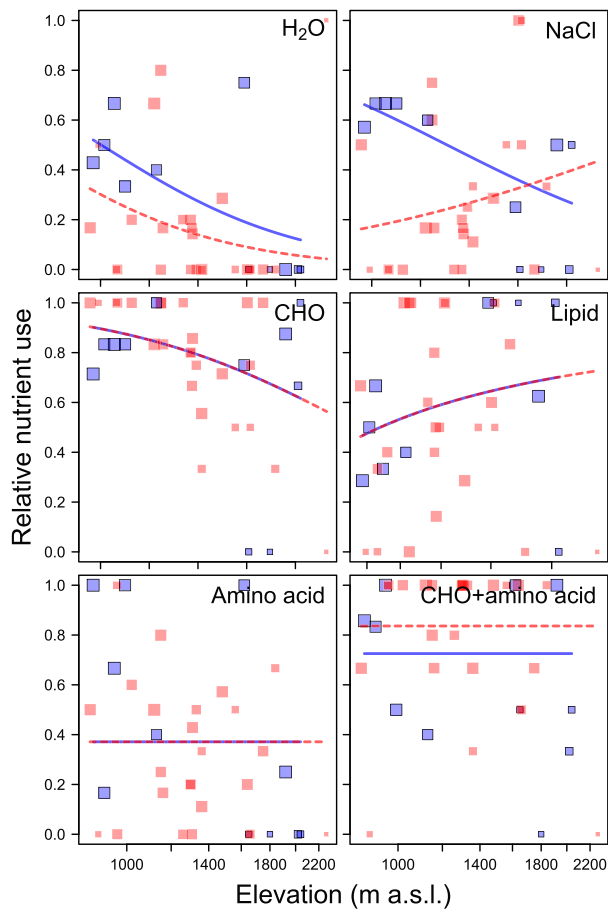**$P < 0.01$; *$P < 0.05$; (*)$P < 0.10$.

Figure 3 Relative recruitment of ants (in relation to the most attractive nutrient) at nutrient baits as a function of elevation and land use (blue, natural habitats; red, managed habitats) at sites on Mt Kilimanjaro, Tanzania. The size of the squares is proportional to the number of observations of nutrient use per study site (smallest square = 1 observation; largest square = 27 observations). Lines show predictions of the mean relative recruitment of ants at nutrient baits derived from generalized linear models. CHO, carbohydrates.

Differences between observed nutrient space and null model expectations increased with increasing species richness of communities in natural but not in managed ecosys-

tems (Fig. 4). In natural ecosystems, communities with low numbers of ant species used a relatively low number of nutrients, while diverse species communities used a large variety of different nutrients, an effect that remained consistent even after controlling for differences in species occurrences among study sites. In managed ecosystems, the nutrient space was broader than in natural habitats at low levels of species richness and narrowed with increasing species richness.

DISCUSSION

Despite the biochemical diversity of living organisms and environmentally driven spatial variation in the availability of nutrients, there have been few studies on the identity and number of nutrients limiting consumer communities along environmental gradients. Our study demonstrates that the use of different nutrients by ant communities varies strongly with changes in abiotic and biotic conditions along elevational and land-use gradients on Mt Kilimanjaro. The high recruitment of ants at nutrient baits, as measured in this study, suggests nutrient limitations in ant populations (Kaspari *et al.*, 2008, 2009). We surmise that ant communities in the multiple environments along the climatic and land-use gradients of Mt Kilimanjaro are limited by different types and numbers of nutrients.

Nutrient use along environmental gradients

Carbohydrates and lipids are the most important energy sources for animals (Hickman *et al.*, 2008). We found that ants were highly attracted to carbohydrates across the distinct ecosystem types along the gradient, which differed strongly in abiotic and biotic conditions, i.e. from the hot savanna and maize fields to the cold and humid montane forests. Ants were more attracted to carbohydrates than to other nutrients, including amino acids, a finding that corresponds well with recent food-choice experiments showing that ants in most cases self-select a carbohydrate-biased diet by actively regulating protein–carbohydrate intake (Dussutour & Simpson, 2009; Cook & Behmer, 2010; Cook
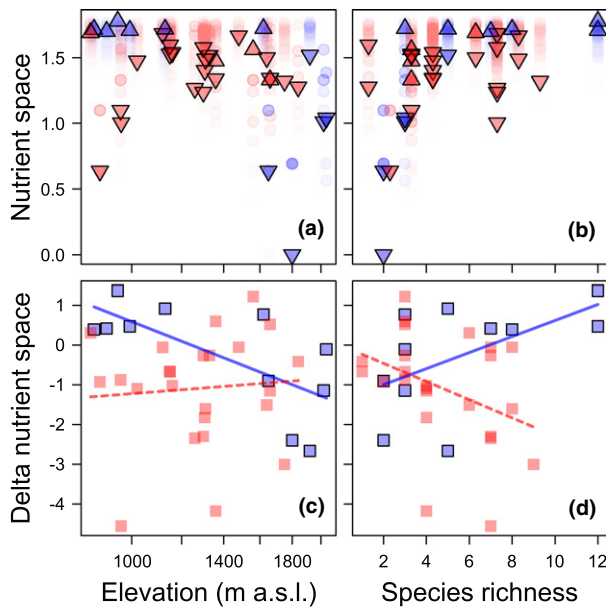
Figure 4 Observed (triangles) and null model predictions (smooth point scatter) of the nutrient space used by ant communities in natural (blue) and managed (red) ecosystems, (a) along the elevational gradient and (b) in relation to species richness, at sites on Mt Kilimanjaro, Tanzania. If the observed nutrient space was broader than the mean of null model predictions the triangle points upwards; if it was narrower the triangle points downwards. Below, standardized differences between observed and predicted (by null models) nutrient space are regressed on (c) elevation (ANOVA, interaction of elevation × land use, $P = 0.09$) and (d) species richness (ANOVA, interaction of elevation × land-use, $P < 0.01$).

et al., 2012); high-protein diets are detrimental for ant workers and colonies (Dussutour & Simpson, 2012).

Interestingly, the relative use of carbohydrates declined in high-elevation habitats while the use of lipids increased. Because lipids are important components for thermal isolation and energy storage, particularly in times of low food availability (Heinze *et al.*, 2003), they may be more important at higher elevations where temperatures are low and food resources are relatively more scarce. Another reason for the increasing importance of lipids at higher elevations could be that communities at higher elevations become increasingly dominated by predatory species (Fig. 2). Along the Mt Kilimanjaro elevational gradient, the proportion of predators increased to 95% in natural habitats and up to 55% in managed habitats. Wilder *et al.* (2013) showed recently that arthropod food webs become increasingly lipid-limited at higher trophic levels, suggesting an increasing importance of additional lipid-rich food for higher trophic level consumers.

NaCl is composed of two elements, sodium (Na) and chloride (Cl), from which the former is often the nutrient targeted by foraging ants (Kaspari *et al.*, 2008; Dudley *et al.*, 2012). In animals, sodium is an important element of cell membrane pumps maintaining osmotic balance and

membrane voltage, and thereby muscle activity and functionality of the nervous system. Kaspari *et al.* (2008) demonstrated continental-scale gradients in the limitation of ant communities by sodium, reflecting declines in aerosol deposition of sodium from the coast (with the ocean being a source of sodium) to inland ecosystems. Because Mt Kilimanjaro is situated approximately 250 km from the ocean, the concentration of sodium in rain is low (Schrumpf *et al.*, 2006) and the NaCl demands of ant communities were therefore expected to be high. Indeed, ant communities in several ecosystem types, particularly the ant communities in the savanna, were highly attracted to NaCl baits. However, this was not the case for communities in managed habitats at lower elevations. One possible explanation for the reduced NaCl use in managed systems could be that irrigation practices on some managed sites in the warm and dry lowlands elevate salt concentrations in the soil (Wakatsuki & Mizota, 1992), although we did not explicitly test this hypothesis.

In contrast to animals, cell membrane pumps of plants are potassium (K) based and, consequently, sodium concentrations in plants are about 100–1000 times lower than in animals. Herbivores (or animals feeding mainly on plant-derived products such as floral nectar and honeydew) often complement their diet with alternative sodium sources (Dudley *et al.*, 2012). In this respect, an observed decline in the use of NaCl with elevation in natural habitats and an increase in managed habitats fits well with the changes seen in the proportion of trophobiotic (honeydew-feeding) ant species within the communities (Fig. 2), which decreased in natural ecosystems and increased in managed ecosystems along the elevational gradient.

After controlling for the possibly confounding effect of water use (in which some nutrients were dissolved), we found no evidence for changes in amino acid use along the elevational gradient or between land-use regimes. Use of amino acid baits was generally low relative to the other nutrient resources. In contrast to pure amino acid baits, baits with a mixture of carbohydrates and amino acids were highly attractive to ants. However, use of the CHO–amino acid mix baits reflected patterns of recruitment by ants at pure CHO baits (linear regression, $r^2 = 0.92$; relative preferences for CHO–amino acid = 0 + 0.99 × relative preferences for CHO, $P < 0.01$), suggesting that the CHO component rather than the amino acid component determined the attractiveness of CHO–amino acid baits. The low attractiveness of pure glutamine baits (the amino acid used in our experiments) for ant communities corresponds with results of previous studies reporting that pure solutions of glutamine or other amino acids are not attractive to ants (Blüthgen & Fiedler, 2004), even though this effect may vary among species (Kay, 2002). Future studies should test different types and combinations of amino acids and analyse the response of ants to natural, complex protein mixes along elevational and land-use gradients (Dussutour & Simpson, 2012; Kaspari *et al.*, 2012). Water makes up *c.* 95% of the body mass and therefore is an essential nutrient for insects. Ant use of H_2O

baits was highest at low elevations and declined with increasing elevation. This pattern mirrors the climatic conditions on Mt Kilimanjaro, where precipitation is low at low elevations and increases up to mid-elevations (i.e. the upper limit of ant occurrences at baits), creating a savanna habitat at the base of the mountain (Hemp, 2006b). For plant populations in temporarily dry and relatively hot habitats like savanna, water availability is a crucial factor limiting plant productivity (Mueller *et al.*, 2012). Our results suggest that water scarcity in these ecosystems also directly or indirectly limits organisms of higher trophic levels in food webs (Western, 1975; Hopcraft *et al.*, 2010), like ant communities, even though these might have access to ground water via trophobiosis (Hölldobler & Wilson, 1990).

Species richness and nutrient space use along environmental gradients

In agreement with regional and global models identifying temperature as an important predictor of ant species richness (Kaspari *et al.*, 2004; Sanders *et al.*, 2007; Dunn *et al.*, 2009; Machac *et al.*, 2011), we found that the species richness of ants attracted to baits declined with increasing elevation. Interestingly, however, this pattern was found only in natural habitats; habitats that were managed or disturbed by humans exhibited a hump-shaped relationship, with species richness peaking at approximately 1200–1300 m a.s.l. and with lower levels of species richness at both the base of the mountain (860 m) and at higher elevations. No ants were found at elevations above 2270 m a.s.l.. We suspect that the drop in ant species numbers in managed habitats at the base of the mountains is caused by an interaction of climate and agricultural practices. In the maize fields in the lowlands of Kilimanjaro, nearly 100% of the above-ground plant biomass is harvested once a year, perhaps restricting the amount of resources at the base of the food chain. In addition, before planting fields are ploughed, which probably disturbs or destroys the nesting sites of ground-nesting ant species (Perfecto, 1991). These disturbances interact with a hot and dry climate throughout more than half the year after harvest, which may allow only a few opportunistic ant species to persist. In contrast, in the savanna, where climatic conditions are similar, a large variety of plants, including grasses (which are important food resources for seed-harvesting ants), herbs and trees (which are important for trophobiotic ants) are available throughout the year and provide complex food resources and shelter from extreme climatic conditions. These conditions may allow the persistence of higher population densities and, in turn, more species of ants (as the 'more individuals' hypothesis predicts; Kaspari *et al.*, 2000).

The nutrient space used by ant communities in natural habitats broadened with increasing species richness. The results from natural habitats support the niche complementarity hypothesis, i.e. that due to the complementarity of dietary niches of coexisting species (Sinclair *et al.*, 2003) ant communities with more species are predicted to forage for a broader spectra of nutrients than species-poor ant communities. Other studies have demonstrated positive correlations between species richness and the diversity of resources used by consumer communities (Bruno & Cardinale, 2008; Cardinale *et al.*, 2012). However, we validated this relationship with food resources analysed at the level of the basic components, i.e. individual nutrients. As multiple environmental conditions change in parallel with changes in species richness with elevation (Körner, 2007), it could be the case that other abiotic or biotic factors determine the size of the nutrient space. Experimental studies, particularly studies manipulating the number of species, are needed to falsify the relationship between species richness and the diversity of nutrients used by consumer communities.

A positive relationship between nutrient space used by ant communities and species richness should be expected only in communities composed of specialized species. In a community of nutrient generalists with heterogeneous diets (e.g. as a function of heterogeneous environments), the exploited nutrient space should be relatively broad at low levels of species richness but remain relatively unaffected with increasing species numbers. In accordance with this theoretical expectation, in managed habitats, where the proportion of generalists was higher than in natural habitats, no positive trend (but a slightly negative trend) between nutrient space and species richness was observed.

Human population growth and the needs for additional agricultural land have led to extensive destruction and fragmentation of natural habitats at the lower elevations of Mt Kilimanjaro (Hemp, 2006b). Therefore, even though we regard the savanna and submontane forest habitats of the lower elevations as natural with respect to the local habitat conditions, the species richness and structure of the ant communities may still have been influenced by land use at larger spatial scales. We therefore consider the higher species-richness and nutrient-use diversity patterns found in natural habitats to be rather conservative estimates of those parameters that could possibly be found under fully undisturbed conditions.

Methodological caveats

In this study, we analysed general patterns in nutrient use in ant communities along an extensive environmental gradient. These data are an important step towards connecting the detailed experimental data derived in the field of nutritional ecology over the past several years with the complexity and environmental variation of natural ecosystems. However, we are aware that our interpretations of the results presented here rest on a few caveats. First, because of the large number of study sites and the number of nutrient types tested (most experimental studies concentrate on ≤ 3 nutrient types), the relatively high effort needed to reach many of the high-elevation sites, and unpredictable climatic conditions that often forced us to skip and repeat experiments, the sampling intensity per site was lower than

some ant ecologists would prefer, i.e. we could only conduct one sampling round per study site and each nutrient type had only five replicates per site. However, the low sampling intensity per site is assumed to affect the error in the data (i.e. the unexplained variation), thereby increasing type II error rates (the failure to reject a false null hypothesis), but is unlikely to explain the significant trends that were detected in our analyses. In addition, we are aware that not all of the ant species that occur at any given site respond to liquid resources placed on the soil surface in 50-mL Falcon tubes. However, we have no reason to assume that the selectivity in sampling is directed towards species belonging to a particular functional group: depending on the habitat, predators and trophobionts were regularly sampled in addition to generalists. Furthermore, the proportion of functional groups in different habitats on Mt Kilimanjaro reflected the patterns observed in other myrmecological studies quite well (e.g. Kaspari et al., 2008).

Second, ant species were not collected continuously from nutrient baits but after 2 h. This was done in order to not disturb the recruitment of ants to the nutrient baits. Because of this long time period, dominant ant species (which are often trophobiotic) may have replaced subdominant ant species from some nutrient baits (Sanders & Gordon, 2003; Parr et al., 2005), so subdominant ant species may be underrepresented in some of our samples. However, even though this may have influenced the distribution of functional groups along elevational gradients it should only have had minor effects on the patterns of relative nutrient use identified in this study. Additionally, we repeatedly observed subdominant ant species in the resource tubes in the presence of dominants. The subdominant species were concentrated at the openings of the 50-mL bait tubes (the inner parts) while the dominant species mostly collected nutrients directly from the cotton ball soaked with the nutrient solution.

Third, we assumed that nutrient-use patterns identified by bait experiments are indicative of nutritional limitations in ant communities: we expected that, if ants were attracted to one particular nutrient, that resource was 'limiting' in the environment because, if it was readily available, the ants would not visit the tube with that nutrient as intensively. This relationship, which is underscored by extensive studies in Neotropical (Kaspari et al., 2008, 2009, 2012) and temperate ecosystems (Fowler et al., 2014), should ideally also be falsified for the variety of ecosystems on Mt Kilimanjaro within which our study was conducted.

CONCLUSIONS

Over the past decade, the field of nutritional ecology has blossomed (Sterner & Elser, 2002; Behmer, 2009; Raubenheimer et al., 2009; Wilder et al., 2013). Most of the research has been based on laboratory experiments on single species that enable a detailed understanding of biochemical processes and provide insights into how individual nutrients determine ecological patterns from the level of individuals to populations and species. Our study complements this work by expanding the scope from local to geographical scales and from the species level to the level of entire communities. Future studies should try to link nutritional states and demands of individuals and populations to community-level responses in space and time. This may require the development of complex theoretical models to be falsified by a combination of manipulative experiments and field data derived from natural communities in variable environments.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Map of the study sites within the study area on Mt Kilimanjaro, Tanzania.

Appendix S2 Data on the ecosystems studied on Mt Kilimanjaro, Tanzania, and data on the sampling times at the study sites.

Appendix S3 List of the ant morphospecies collected at nutrient baits along an elevational gradient on Mt Kilimanjaro, Tanzania.

BIOSKETCH

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Author contributions: M.K.P, N.J.S. and I.S.-D. designed the study; M.K.P., A.M. and J.R. collected the data; M.K.P. analysed the data and wrote the first version of the manuscript; all authors contributed to the writing of the final manuscript.

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