Exploring Nutrient Availability and Herbivorous Insect Population Dynamics Across Multiple Scales

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Abstract

1. Nutrient composition varies greatly across landscapes, which in turn influences the spatiotemporal variation and dynamics of populations. Yet few studies have investigated this pattern on multiple scales.
2. We tested how nutrient limitation affects herbivore populations across spatial scales from individual behavior to landscape level population dynamics using Australian plague locust, Chortoicetes terminifera (Walker), field populations and long-term survey data from across their range.
3. At the individual level, juvenile locusts selected for a carbohydrate-biased intake target of 1 protein (p) 2 carbohydrate (c) and had the highest growth and shortest development time when fed artificial diets with that same 1p:2c ratio over the duration of the 5th (final) juvenile instar.
4. At the field level, locusts kept in field cages with protein-biased plants redressed their nutritional imbalance by selecting very carbohydrate-biased diets (up to a 1p:20c ratio) for more than a week after being removed from the protein-biased environment. Once the deficiency was met, locusts returned to the 1p:2c intake target.
5. Going up to the landscape level, outbreaks were negatively correlated with soil nitrogen and showed a non-linear relationship with soil phosphorus peaking at approximately 4% soil phosphorus. We disentangled the relationship between mean annual precipitation and soil nitrogen through leveraging both comprehensive locust surveys and remotely sensed soil and weather data that spanned decades.
6. This paper is the first to integrate lab, field, and remote sensing approaches to demonstrate the importance of nutrient balancing and acquisition across scales for herbivores. Specifically for locusts, we show that low nitrogen environments promote outbreaks, likely by reducing plant protein to carbohydrate ratios. Incorporating soil quality data into locust plague forecasting models can help increase accuracy of predictions.

# 1. INTRODUCTION

Plant nutrients are usually more variable than animal body composition, which can make it challenging for herbivores to acquire an optimal nutritional balance (Elser et al., 2000). Nutrient balance affects individual performance (Batzli, 1986; Bernays et al., 1994) and, by extension, influences population dynamics (Elser et al., 2000). One way many primary consumers achieve an optimal blend of nutrients is through selecting different host plants or plant parts to reach a specific amount and ratio, termed the intake target, IT (S. J. Simpson & Raubenheimer, 2012). For example, giant pandas (Ailuropoda melanoleuca) synchronize their migration and reproduction cycles with seasonal nutrient availability between different bamboo species (Nie et al., 2015). While studies have investigated this trend between available nutrients and animal populations, they usually happen at one scale and biological level. For example, much grasshopper nutrition research has been done at the organismal or local population level using lab and/or field studies (Behmer, 2009; Cease, 2024; Le Gall, Overson, et al., 2019), with a few separate larger scale modeling endeavors (Welti, Roeder, et al., 2020; Zhou et al., 2019). To our knowledge, no study has integrated individual herbivorous animal nutrition to continental level patterns in nutrient availability. Here we work across spatial scales, bridging field observations and cage manipulations with continental-scale insect pest outbreak modeling to see how the animal-nutrient relationship changes across multiple scales.

Food quality has long been considered an important predictor of animal population dynamics (Andersen et al., 2004; White, 1993). Phosphorus and nitrogen commonly limit primary production in ecosystems, either separately or as co-limiting factors (Tyrrell, 1999; Vitousek et al., 2010), and are often investigated as limiting factors for higher trophic levels to determine the consequences to animal growth and reproduction (Andersen et al., 2004). For example, bluegrass fields supplemented with high quality (e.g. increased nitrogen) food pellets increase vole (Microtus ochmgaster) densities compared to control fields by supporting higher adult survival, increased breeding, and growth rates (Cole & Batzli, 1978). Less phosphorus in a dryland insect herbivore’s (Sabinia setosa) host plant, velvet mesquite (Prosopis velutina), leads to individuals having decreased RNA content (slower growth) and lower abundance (Schade et al., 2003). More broadly, nitrogen and phosphorus limitation has been shown in mammals (Randolph et al., 1995; White, 1993), birds (Forero et al., 2002; Granbom & Smith, 2006), and insects (Floyd et al., 1996; Huberty & Denno, 2006; Marsh & Adams, 1995; Perkins et al., 2004) which is discussed further in (White, 2008) and (Andersen et al., 2004). However, not all animal populations respond positively to increasing environmental nutrients. For example, studies from across the globe reveal that the abundances of many grasshopper species are positively (Joern et al., 2012; Ozment et al., 2021; Welti, Prather, et al., 2020; Welti, Roeder, et al., 2020; H. Zhu, Nkurunziza, et al., 2020), negatively (Lawton et al., 2020; Ritchie, 2000; H. Zhu, Li, et al., 2020; Y. Zhu et al., 2019), or not related (Heidorn & Joern, 1987; Jonas & Joern, 2008; Lenhart et al., 2015; Ozment et al., 2021) to plant nitrogen concentrations (reviewed in Cease, 2024). Can the relationship between herbivore abundance and environmental nutrients be explained by approaches that consider nutritional physiology at the organismal level, in addition to population and landscape ecology?

One excellent study system to understand the complex relationship of nutrient availability and population dynamics is a subset of grasshoppers called locusts (Cullen et al., 2017; Uvarov, 1977). Locusts undergo massive population irruptions known as plagues that pose significant issues for global food security (Cease et al., 2015). For example, the 2010-2011 Australian plague locust (Chortoicetes terminifera) plague could have caused AUD$963 million in crop damage if not controlled (Millist & Abdalla, 2011). In contrast to the nitrogen limitation paradigm (White 1993), locusts frequently show carbohydrate limitation. Many locust species, including C. terminifera, will select plants with a low protein:carbohydrate ratio and eat about double the amount of carbohydrate relative to protein when given a choice of artificial diets (Behmer, 2009; Brosemann et al., 2023; Lawton et al., 2021; Le Gall, Overson, et al., 2019). Their high energy, relative to protein, demand is heightened by their impressive long-distance migration. Marching bands of South American locusts (Schistocerca cancellata) eat predominantly from dishes containing carbohydrates and largely ignore dishes containing protein (Cease et al., 2023), and have the highest survival and lipid gain when eating carbohydrate-biased plants (Talal et al., 2020). As adults, flight increases carbohydrate, but not protein, consumption in migratory locusts (Locusta migratoria) and locusts eating carbohydrate-biased diets fly for longer times (Talal, Parmar, et al., 2023). At a local scale, high-use agricultural areas that decrease soil and plant nitrogen promote Oedaleus locust outbreaks in Senegal and China (Cease et al., 2012; Giese et al., 2013; Le Gall, Word, et al., 2019; Word et al., 2019). Less is known about the relationship between plant phosphorus and locust populations, but grasshoppers and locusts can detect and regulate phosphorus during ingestion and post-ingestively to maintain homeostasis (Cease et al., 2016; Zhang et al., 2014). Long-term laboratory rearing studies show negative effects of too little or excess dietary phosphorus, though short-term restrictions to low or high phosphorus diets appear to have no effects (Cease et al., 2016). Field measurements of Oedaleus decorus asiaticus in China show that locusts increase phosphorus excretion with increasing plant phosphorus content, suggesting that phosphorus may not be limiting in the Inner Mongolian Steppe for this species (Zhang et al., 2014). Understanding the multi-scale flow of nitrogen and phosphorus from soils to continental scale population dynamics may reveal a connection between soil quality and locust plagues in Australia and further elucidate the pattern globally (Cease, 2024; Le Gall, Overson, et al., 2019).

The arid interior of Australia is a highly variable environment (Morton et al., 2011) characterized by the Resource-Pulse Paradigm (Noy-Meir, 1973, 1974; Whitford & Whitford, 2002). Many Australian animal and plant populations irrupt following periods of favorable climatic conditions. For example, preceding vegetation growth is an important factor for Australian plague locust population outbreaks (Lawton et al., 2022). In addition to climatic variability, Australia is marked by poor natural soil fertility (Morton et al., 2011; Orians & Milewski, 2007). Nutrients such as nitrogen and phosphorus are in short supply in unmodified soils. Subsequently, many native animals and plants are adapted to environments low in soil nutrients (Orians & Milewski, 2007). Australia has several locust species, but the Australian plague locust is the most widespread and economically damaging (Hunter et al., 2001). As with many dryland animals, numerous studies have shown the relationship between climatic conditions and Australian plague locust population outbreaks (Clark, 1974; E. Deveson & Walker, 2005; Farrow, 1982; Key, 1945; Wang et al., 2019), however few have investigated the relationship between soil and plant nutrients and this species’ outbreaks.

In this study, we investigate the across scale relationship between soil and plant nutrients and animal populations using Australian plague locusts. We have three specific questions and subsequent predictions across scales:

1. What are the nutritional preferences of individuals from gregarious Australian plague locust field populations? We expect individuals to be carbohydrate biased based on their active lifestyle, and to keep a constant intake target between populations.
2. What happens to Australian plague locusts when constrained to high nitrogen environments? We expect locusts to select particularly carbohydrate biased diets to redress a protein : carbohydrate imbalance in their local environment.
3. Can we use soil nutrients as a predictor of Australian plague locust nymph outbreaks at the continental scale?

At larger scales, and accounting for climatic factors, we expect outbreaks to be associated with low nitrogen areas. Since terrestrial herbivores can require 5-50 times more nitrogen than phosphorus (Elser et al., 2000) and locusts likely have a higher capacity to post-ingestively regulate phosphorus in ranges found in nature (Cease et al., 2016; Zhang et al., 2014), we expect nitrogen to be a stronger predictor of populations at a continental scale as compared to phosphorus.

# 2. METHODS

## 2.1 Field site and animals

Lab-based and field cage experiments were conducted at the Trangie Agricultural Research Centre of the Department of Primary Industries in Trangie, New South Wales, Australia. We used field populations of the Australian plague locust, which prefers open grassy areas and is a grass generalist (Key, 1945). Solitarious populations of this locust species maintain their intake target closely regardless of spatiotemporal changes (Lawton et al., 2021). We carried out these studies in November-December 2015, during the final nymphal and early adult stages of the spring Australian plague locust generation.

## 2.2 Nutritional target and performance curve using synthetic diets

To control the nutrient availability within the experiments, we used isocaloric artificial diets made up of 42% macronutrients (differing ratios of protein and carbohydrates), 32% cellulose, and 4% of salt, sterols and vitamins. This artificial diet mixture has been used with this species (Clissold et al., 2014) and was developed by (S. J. Simpson & Abisgold, 1985) from (Dadd, 1961). We dried diets to a constant mass at 60°C before weighing the diets before and after all experiments.

We collected fourth and fifth instar nymphs from outbreaking populations on Nov. 12, 2015 from two locations: near Mendooran (31°40.791’ S, 149°04.209’ E) and Guntawang (32°23.988’ S, 149°28.649’ E), New South Wales. The Mendooran population was forming marching bands while the Guntawang population was at high density but was not actively marching during the collection period. Both populations were collected along dirt roads flanked by livestock grazing pastures containing a mix of grasses and forbs. We transported locusts back to the Trangie Agricultural Research Centre and kept them in large plastic bins with holes along with an assortment of grasses and forbs cut from the same field locusts were collected until experiments were started. For both experiments, locusts were housed individually in plastic cages (ca. 18 cm L x 12 cm W x 4 cm H) with small holes for ventilation. Each cage had two (nutritional target) or one (performance curve) artificial diets, a water tube capped with cotton, and a perch.

### 2.2.1 Nutrition target (choice diets)

We ran this experiment to determine the preferred dietary p:c ratio of Australian plague locust field populations. We selected equal numbers of males and females from both Mendooran and Guntawang populations and individuals that had no visible wing bud separation to ensure they were early in the fifth instar. Locusts were heated with incandescent light bulbs on a 14:10 light:dark cycle. The average daytime and nighttime temperatures in the cages were 25.5°C and 23.4°C respectively, recorded from an iButton (Maxim). We randomly assigned 80 locusts (20 individuals per diet pair per population) to one of two treatment groups receiving pairs of either 7p:35c & 28p:14c or 7p:35c & 35p:7c (% p : % c by dry mass). We used two different pairs to ensure that the selected p:c ratio was not the result of eating randomly between the two dishes. We let locusts eat from the dishes for three days (Nov. 12-15, 2015) and calculated the mass of protein and carbohydrate each locust ate by subtracting the final dish weights from the initial dish weights.

### 2.2.2 Performance curve (no-choice diets)

We ran this experiment to determine the growth rate and development time to adulthood of Australian plague locust juveniles in response to different dietary p:c ratios. We isolated fourth instar nymphs from the Mendooran population and housed them in individual cages. Each day, we retrieved individuals that had molted into the fifth (final) stadium to incorporate into the experiment. We added an assortment of grasses and forbs collected from grazing pastures in the Research Centre to the cage of fourth instars daily. On day one of the fifth instar, locusts were randomly assigned to one of four artificial diet treatment groups: (7p:35c, 14p:28c, 21p:21c, or 35p:7c), with 18 locusts per treatment (similar numbers of males and females for each group). Fifth instar locusts were started on diets between Nov. 15-19 and we ended the experiment on Dec. 3, 2017. If locusts molted to adults before that point, we recorded the adult mass on the day they molted and ended that individual trial. We fed locusts their assigned diet ad lib such that there was always food available. We changed diets every three days until locusts molted to adults or until the experiment ended. Locusts were heated with incandescent light bulbs on a 14:10 Light:Dark cycle. The average daytime and nighttime temperatures in the cages were 26.2°C and 23.9°C, respectively, recorded from an iButton.

## 2.3 Field cage experiments

We ran this experiment to test the effects of different host plant nitrogen contents on locust nutrient balancing, growth, and survival. This experiment was conducted in a research field at the Trangie Research Centre that is used mainly for livestock grazing and had no history of fertilization. We marked nine 7 m x 7 m plots with 2 m alleyways between each plot. Each of the three nitrogen addition levels (0, 87.5, and 175 kg N/ha) was randomly assigned to three of the nine plots. We chose 175 kg N/ha as the upper range because this is similar to the fertilization rates of most crops. We added the fertilization treatment one time, on Nov. 2, 2015, in the form of urea, two weeks before a significant rainfall (ca. 60 mm). This treatment regime allowed us to see how increasing levels of nitrogen affected nutrient redressing and overall performance of locust populations through time.

We built 0.75 x 0.75 x 0.75 m cages with four steel fence posts and aluminum mesh. We secured the mesh to the ground by partially burying it and nailing thin slats of wood over the mesh edges. Before adding locusts to the cages, we removed any plant litter and arthropods (mostly spiders and locusts) so we could more easily count the locusts and limit predation. We added 20 4th instar locust nymphs to each field cage on the evening of November 13th and morning of November 14th. We randomly selected these nymphs from the Mendooran population we collected on November 12th (see synthetic diet methods section above). Each fertilized plot had four cages, for a total of 12 cages per fertilization level, and 36 cages and 720 locusts in total. We checked the cages every 4-5 days and removed any spiders.

We measured plant diversity and abundance in each locust cage and outside the cage within each fertilized plot. We also measured ground, litter, manure, and plant cover in each of the nine plots by randomly tossing three 0.25 x 0.25 m quadrats within each of the nine plots. We took these ground cover and biodiversity measurements at the beginning and end of the experiment. We took plant leaf samples for nutrient analyses from each cage three times during the experiment on November 11th, November 25th, and December 1st, 2015. Plant species with resulting carbon, nitrogen, digestible carbohydrate and protein content as well as soil nitrogen (from 0-10 cm cores) can be seen in supplementary table 1. Plant species ground cover for each cage can be seen in supplementary table 2. The relationship between soil NO3 and NO4 within cage plots and cage plant carbohydrates and proteins are visualized in supplementary figure 1.

To test how the nitrogen fertilization treatments affected the nutritional status of the locusts, we collected 20 locusts from control and high N treatment cages (40 individuals in total) on November 23, 2015. We housed them in individual cages and gave them the choice of a low protein: carbohydrate diet and a high protein: carbohydrate diet. We changed their diets on days one, two, four, and six and ended the experiment at day nine. To ensure an adequate sample size, we included data from any individuals that died (approximately 60% mortality) before the experiment was completed and individuals which molted during the experiment. We calculated the mass of protein and carbohydrate locusts ate over each interval. This allowed us to see the nutritional redressing of individuals.

We collected all remaining field-cage locusts on Nov. 28, 2015 and recorded developmental stage and body mass. We calculated surviving proportion for each cage accounting for the locusts we removed for the nutritional status experiments (surviving proportion = # live locusts/(initial # added-# locusts removed for secondary experiment)). We calculated the proportion molted to adult of the surviving locusts (# adults/# live locusts).

## 2.4 Correlating historical nymphal outbreaks with soil nitrogen and phosphorus

To understand the relationship between soil nutrients and locust outbreaks, we used the Australian Plague Locust Commission’s (APLC) long-term locust survey dataset (T. Deveson & Hunter, 2002) and the Soil and Landscape Grid Australia-Wide 3D Soil Property Maps (Grundy et al., 2015).

### 2.4.1 Locust outbreak data

Australian plague locust data from 2000 - 2017 (~ 190,000 records) were used in this study. This database contains georeferenced points with a categorical ordinal variable 0-4 to represent approximate nymph densities. The value ranges for nymphs are: 0 = nil, 1 = < 5 m2, 2 = 5-30 m2, 3 = 31-80 m2, and 4 = > 80 m2. Population outbreaks are characterized by very high densities of gregarious nymphs (up to 1000 m2). We focused on nymphs as this life stage cannot fly and can be used to assess prior local habitat conditions. While the database extends into the 1980s, we only used data collected between 2000-2017 as this period overlapped with the soil grid data which was released in 2015. Since climatic conditions and migration patterns are drivers in outbreak occurrence (Lawton et al., 2022; Veran et al., 2015) they likely mask the relationship between static environmental variables like soil nutrients. To account for this variability, we spatially aggregated the survey dataset to a 1 km2 x 1 km2 grid as can be seen in supplementary figure 2. This allowed us to model how often locust outbreaks occur in grid cells rather than the actual outbreak. We used a 1 km2 x 1 km2 grid as this reflects the estimated maximum dispersal distance from hatching to 5th instar (Hunter et al., 2008). We counted the number of outbreaks (APLC nymph density code 4), the number of nil records (APLC nymph density code 0), and total number of survey observations. This resulted in approximately 12,000 grid cells for the final dataset. Overall APLC survey point distribution can be seen in Figure 1A.

### 2.4.2 Soil grid of Australia data

The soil and landscape grid of Australia is a modeled raster of 12 soil variables at a 90 m2 resolution. These soil characteristics are available at multiple depths from surface to two meters below the surface. These depths and methodology are consistent with the specifications of the Global Soil Map project (http://www.globalsoilmap.net) (Grundy et al., 2015). We extracted this data from Google Earth Engine (Gorelick et al., 2017). In this data set both phosphorus and nitrogen units are the mass fraction of total nutrient in the soil by weight. We took the average of nitrogen and phosphorus of the top two depths (0 cm – 5 cm and 5 cm and 15 cm) as these are most relevant to nutrient content of grasses and forbs. Then, for each APLC survey grid we calculated the mean mass fraction of nitrogen and phosphorus in the soil by weight to a 15 cm depth. Spatial distribution of soil nitrogen and phosphorus can be seen in Figure 1B and 1C respectively.

## 2.5 Statistics

All statistics were conducted with a generalized additive mixed modeling (GAMM) approach when possible. This allowed us to test for non-linear and linear trends in the dataset and specify the hierarchical nature of the data. All statistics were conducted in R (R Core Team, 2021) in the tidyverse framework (Wickham et al., 2019). GAMs were constructed with mgcv (Wood, 2017) and validated with gratia (G. L. Simpson, 2023). Post hoc comparisons were done with multicomp (Hothorn et al., 2008). Spatial data management was done with the following packages: raster (Hijmans et al., 2015), SpatialEco (Evans & Murphy, 2023), rgdal (Bivand et al., 2015), and sf (Pebesma, 2018). APLC survey grids and final maps were produced in ArcGIS.

### 2.5.1 Intake Targets

To determine intake targets, we constructed GAM (family: Multivariate Normal Distribution, Link: Identity) with the following variables when possible: diet pairing (factor), locust sex (factor), time period interval (integer), locust initial weight (numeric) following roughly the procedure found in (Lawton et al., 2021). We selected the inclusion of locust weight as either a non-linear or linear effect via Akaike information criterion (AIC), AIC adjusted for small sample size (AICc), and Bayesian information criterion (BIC). If weight was not an important variable, it was removed entirely from the model.

### 2.5.2 Field population

We calculated intake targets as discussed above. To see the impact of confined diet treatments, we constructed GAMs (family: gaussian, link: identity) with the following variables: treatment (factor), locust sex (factor), population (factor), and locust initial weight (numeric).

### 2.5.3 Field Cage

We assessed plant nutrients with a GAMM (family: Multivariate Normal Distribution, link: identity) and included the following variables: plant carbohydrate (numeric, dependent), plant protein (numeric, dependent), treatment (factor, independent), cage (factor, random effect), plot (factor, random effect), and plant species (random effect). Redressing intake targets were conducted as discussed above (section 2.5.1). To see the difference between physiological performance and fertilizer treatments, we constructed GAMMs (family: Scaled T distribution, link: identity) for final locust mass. The independent variables in all models were treatment (factor), sex (factor), a two-dimensional smoother of available protein and carbohydrate, and cage number as a random effect. For both final adult proportion and survival proportion, we constructed a GAM (Family: Beta, Link: identity) and included the following variables: treatment (factor) and a two-dimensional smoother of available protein and carbohydrate.

### 2.5.4 Historical outbreaks and soil nutrient grid modeling

To relate nymph survey grids to soil nitrogen and phosphorus, we constructed two GAMMs (family: tweedie, link: log) predicting the number of outbreaks (APLC Survey Category 4) and nil observations (category 0). Since both soil nitrogen and mean annual precipitation are highly correlated (Pearson correlation = 0.93) with both variables decreasing going into the arid interior of Australia, we are unable to add precipitation directly to the model as it would bias the results. Instead, we built a comparison model with mean annual precipitation between 2000 and 2017 switched for soil nitrogen. To do this, we calculated the average precipitation between 2000 and 2017 for all survey grids using the European Centre for Medium-Range Weather Forecasts’ ERA5 reanalysis dataset (Muñoz-Sabater et al., 2021). This allowed us to visually compare the effect differences of soil nitrogen and mean annual precipitation on locust outbreaks. In other words, if soil nitrogen and mean annual precipitation were so tightly correlated that the effects are indistinguishable, the modeled results should look very similar. The soil models had the following independent variables: soil nitrogen, phosphorus, latitude / longitude, bioregion, and the number of observations within each grid. For the precipitation model, all variables were the same except mean annual precipitation replaced soil nitrogen and phosphorus. The inclusion of bioregions as a random effect allowed us to account for variation due to vegetation community and soil characteristics. The inclusion of latitude and longitude allowed us to account for spatial autocorrelation (Clayton et al., 1993). Lastly, the inclusion of the total number of observations allowed us to account for sampling intensity biases.

# 3. RESULTS

## 3.1 Field population

### 3.1.1 Choice experiment (nutritional target)

Australian plague locust individuals from the two outbreaking populations regulated to a specific ratio of 1 protein : 2 carbohydrate (Figure 2A, Table 1). Model selection can be seen in Supplementary Table 3. Consumption in the two diet pairings did not differ, indicating non-random feeding (Supplementary Figure 3A, Table 1). While the protein : carbohydrate ratio did not change, females consumed more food than males, likely due to being bigger overall (Supplementary Figure 3B, Table 1, Supplementary Table 3).

### 3.1.2 No choice experiment (performance curves)

Australian plague locusts had higher specific mass growth rates and shorter development times on the 1 protein : 2 carbohydrate (14 protein : 28 carbohydrate) diet as compared to the other diets (Fig. 2B & C, Table 2, Supplementary Table 4).

## 3.2 Field Cage

For the first 11 days of the 14 day field cage experiment, plant protein and carbohydrate contents remained consistently protein-biased for all treatments (Fig. 3A-C, Table 3), and only showed differences in protein content by the last sample period on December 1, which was after the end of the locust cage experiment. Accordingly, there was no effect of fertilizer on locust survival and adult proportion, though locusts in the medium level nitrogen fertilizer treatment were heavier than locusts in the high nitrogen fertilizer treatment (TukeyHSD: difference: -0.043, p-value: < 0.001) (Fig. 3D-F, Table 4). Locusts that were retrieved from field cages after nine days and were given a choice to regulate protein and carbohydrate intake showed a pattern consistent with rebalancing a shortage of carbohydrates (Fig. 4, Supplementary Fig. 4, Table 5). Irrespective of fertilizer treatment group, locusts initially selected very carbohydrate biased diets, but gradually, after 9 days, their trajectory returned close to the predicted intake target of 1p : 2c (Fig. 4, Supplementary Fig. 4).

## 3.3 Locust outbreaks

Australian plague locust outbreaks were negatively associated with soil nitrogen, which supports the hypothesis that nitrogen (in excess) acts as a limiting factor for population upsurges (Table 6, Fig. 5A). Australian plague locusts had a nonlinear relationship with soil phosphorus with outbreaks occurring more often in areas with approximately 4% soil phosphorus and were strongly negatively associated with increasing phosphorus afterwards (Fig. 5B). For both nutrients, the absence models had a very weak relationship with soil nutrient in comparison to the outbreak models, demonstrating little model bias due to APLC survey protocol. There were significant nonlinear relationships between coordinates and the total number of observations in all models (Supplementary Figs. 5 & 6). The relationship between locust outbreaks and mean annual precipitation (e.g. nonlinear) was very different from the relationship with soil nitrogen (e.g. linear; Fig. 5, Supplementary Fig. 7). Soil nitrogen and phosphorus show weak positive correlations with woody vegetation cover, while mean annual precipitation exhibits high variation in its relationship with soil nitrogen and weak correlation with soil phosphorus (Supplementary Figure 8). Thus, the relationship between soil nitrogen and locust outbreaks cannot be fully explained by differences in woody vegetation.

# 4. DISCUSSION

We show that herbivore diet preferences remain consistent between spatial levels, from individual foraging behavior and physiology to large scale population dynamics, with locust populations negatively related to environmental nitrogen. Thus by going across scales, this study shows a consistent pattern of excess nitrogen limiting a pest herbivore and introduces a more nuanced view of phosphorus limitation on herbivore populations. Instead of the broad generalization that animals are always negatively or positively associated with certain nutrients, specific life history traits, such as energetically-costly migration, as well as organism-environment interactions should be considered. For forecasting pest populations dynamics, adding variables describing the nutritional quality of landscapes can inform seasonal scouting surveys. We hope that this study spurs future interest in multi-scale experiments and modeling of nutrient availability with animal population dynamics.

## 4.1 Field populations

Field populations of final instar Australian plague locusts behaviorally regulated to a 1 protein (p) : 2 carbohydrate (c) nutrient ratio, which supported the fastest nymphal growth and the lowest development time to adulthood (Fig. 2B & C), consistent with previous studies (Clissold et al., 2014; Lawton et al., 2021). Locusts are highly mobile (Australian plague locusts can fly up to 500 km in a single night, (E. Deveson & Walker, 2005)) and the demand for energy via carbohydrates and lipids likely increases relative to protein demand during the later life stages of these animals.

Plant nutrient content in the nitrogen fertilization treatments was not significantly different until the last sample period, which likely explains the small effect on locust growth (Fig. 3A-C). Over the experimental period, protein content decreased in unfertilized treatments while both plant protein and carbohydrate remained constant in the fertilized treatments. If we prolonged the experiment, there might have been a noticeable difference in locust survivorship, weight gain, and adult proportion given the shift in nutrients among treatments (Fig. 3D-F).

Importantly, all field cage plants were protein biased (roughly 1p : 1c to 2p : 1c ) as compared to the desired locust intake target of 1p : 2c. When locusts were subsampled from the field cages mid-experiment and given the opportunity to select carbohydrate or protein diets, they selected extremely carbohydrate-biased diets for more than a week. This behavior indicated that locusts in the small field cages were highly carbohydrate-limited, driving them to overeat carbohydrates to redress the imbalance. Interestingly, multiple studies have shown that the Australian nutritional landscape is often too protein-biased relative to what the Australian plague locust prefers (Lawton et al., 2020, 2021). Regardless, populations are still persistent and outbreaks can occur at lower frequencies in these areas (E. D. Deveson, 2013; Key, 1945). How this species can achieve the optimal balance of nutrients within an unfavorable nutritional environment merits further investigation, but may include postingestive regulation and/or large-range foraging. Migratory locusts (Locusta migratoria) can choose microclimates that favor higher efficiency of carbohydrate or protein absorption depending on their host plant and nutritional status (Clissold et al., 2013). For this study, we collected free-living locusts from the same region and a similar environment as where we built the field cages, yet those confined to field cages selected a 10 x decrease in p:c (1p : 20c vs 1p : 2c). This result suggests that free-living locusts are able to persist in high protein regions by foraging over a larger range to seek out pockets of carbohydrate-rich plants and that the limited foraging range of the field cages precluded field-cage locusts from finding sufficient carbohydrates. Similarly, these results suggest that, while Australian plague locusts can persist in low numbers in nitrogen rich regions, those environments are unlikely to support extreme outbreaks due to a limitation of carbohydrate-rich resources. We tested this prediction using historical outbreak and large-scale soil nutrient modeling.

## 4.2 Historical outbreak modeling

This is the first time to our knowledge that terrestrial animal population dynamics have been modeled with nutrients at the continental level, allowing nutrient limitation to be tested at a scale not previously investigated. Locust outbreaks are associated with decreasing soil nitrogen (Fig. 5A), suggesting that nitrogen acts as a limiting factor not due to its deficit (White, 1993) but its excess. Plants growing in high nitrogen environments tend to have high p:c ratios, which force locusts to either undereat carbohydrates (limiting their energy to support growth and migration) or overeat protein (which can be toxic) to acquire sufficient carbohydrates (Behmer, 2009; Cease, 2024). On the other end of the performance curve, Australian plague locusts do have a lower p:c range that limits performance, as shown using artificial diets (Fig. 2B-C). However, our outbreak models found no lower limit (Fig 5A), suggesting that locusts do not often encounter environments where protein deficit limits their growth. This result is consistent with other research that found most grasses across New South Wales tend to have higher p:c ratios than locusts require (Lawton et al., 2021). Even when locusts have access to low p:c plants it is more difficult for them to extract carbohydrate than protein (Clissold et al., 2006), further exacerbating the problem of relative protein excess. Interestingly, this relationship between nitrogen and locusts stays consistent throughout multiple spatial levels which suggests that it scales linearly; an uncommon characteristic in ecology (Levin, 1992; Wiens, 1989). We also show that outbreaks are correlated with a low level of soil phosphorus, however, outbreaks peak at approximately 4%, suggesting that while locusts generally do well in low phosphorus environments, phosphorus deficit can be limiting for locusts in extremely phosphorus poor soils (Fig. 5B). Because Australian soils are characteristically phosphorus poor (Donald, 1964), Australian animals like this locust are adapted to phosphorus poor environments and potentially having too much phosphorus is deleterious (Morton et al., 2011). Locust populations may be more tightly correlated with soil nitrogen than phosphorus because terrestrial herbivores require 5-50 times more nitrogen than phosphorus (Elser et al., 2000), meaning they can more readily balance phosphorus by eating a few foods rich or poor in phosphorus but cannot as quickly regulate protein and carbohydrate energy because they make up the bulk of their required nutrients. Indeed, laboratory studies have revealed that short-term limitations in dietary phosphorus have no apparent impact on grasshopper growth (Cease et al., 2016), suggesting that these mobile herbivores could seek out phosphorus-rich diets intermittently to overcome potential phosphorus limitation in field environments. However, in this study, we only tested this relationship with phosphorus at the continental level; further field and laboratory experiments are needed to explore this non-linear relationship between locust outbreaks and soil phosphorus. While we only looked at nitrogen and phosphorus, it is also important to note that animals require a suite of nutrients. Other nutrients such as potassium and sodium (Joern et al., 2012) warrant further investigation. Comparing locust outbreaks between continents would further show the relationship between nutrient availability and animal population dynamics. One excellent dataset for this would be SoilGrids (https://www.isric.org/explore/soilgrids) which provides soil nitrogen estimates globally at a 250-meter resolution.

Lastly, our results suggest that forecasting efforts for locusts should consider the inclusion of a nutritional landscape quality metric like soil nitrogen. Current forecasting models use climatic data (e.g. rainfall and soil moisture) or vegetation growth data (e.g. normalized difference vegetation index, NDVI) as the major predictors of outbreaks (Cressman, 2013). While these climatic variables are clearly important, adding metrics to quantify the nutritional landscape can help increase forecasting model accuracy in environments with highly variable climates.

## 4.3 Locusts are more likely to be limited by high nitrogen environments than other grasshoppers

A five-decade review of grasshopper responses to plant nitrogen content showed that grasshoppers not classified as locusts have a variation of negative, neutral, and positive responses to increasing plant nitrogen (Cease, 2024). Looking just at field surveys, there are more reports of a negative correlation between plant nitrogen and non-locust grasshopper abundance (17 reports) relative to neutral (6 reports) or positive (9 reports). This pattern corroborates long-term studies showing that dilution of plant nitrogen is correlated with declines of North American grasshopper populations (Welti, Roeder, et al., 2020). Of the studies that report correlations between individual grasshopper species abundance and plant nitrogen, most are from graminivorous (grass-feeding) species (11 reports), with 7 reports from mixed (grasses and forbs) or forb feeders (Cease, 2024). The abundance of non-locust graminivorous species tended to be more positively correlated with plant nitrogen, which would support the hypothesis that grass-feeders are more likely to be nitrogen-limited because grasses tend to have lower p:c ratios than forbs; although this trend was not significant and grass-feeders also regularly showed negative responses to high plant nitrogen. In contrast, there was a consistent negative effect of high plant nitrogen on locust species, regardless of whether they were graminivorous or mixed feeders. Because mass specific protein consumption is highly correlated with growth rate in both lab and field populations, but carbohydrate consumption is highly influenced by the environment (Talal, Harrison, et al., 2023), it is most likely that locusts have similar protein requirements as other non-locust grasshopper species, but have much higher carbohydrate demands, potentially to support migration (Raubenheimer & Simpson, 1997; Talal et al., 2021; Talal, Parmar, et al., 2023). Locusts are able to meet this increased demand for carbohydrate, while keeping protein consumption constant, by eating larger amounts of low p:c plants found in low nitrogen environments. In summary, these studies suggest that nymphal outbreaks of all locust species may be negatively correlated with soil nitrogen across continental scales, but that the correlation between plant nitrogen and non-locust grasshoppers may not be significant or consistent through space and time.

## 4.4 Comparing the relationship between plant macronutrients and herbivore abundance in other taxa

The effect of plant protein and carbohydrate on herbivore populations is predicted to depend on the herbivore’s p:c intake target (IT) relative to its nutritional landscape (Le Gall et al., 2020). If there are sufficient plants on either side of the IT, herbivores can select from between them to achieve their IT. This complementary feeding has been recorded for field populations of blue sheep (Psuedois nayaur) in the Himalayan Mountains (Aryal et al., 2015), Black Howler Monkeys (Alouatta pigra) in Yucatán (Bridgeman, 2012), and other primates (Raubenheimer et al., 2023). There would be a predicted impact on populations if the nutritional landscape were to become more constricted or not overlap with the IT. For example, lab colonies of tobacco hornworms (Manduca sexta larvae) have an IT around 1:1 or sometimes slightly carbohydrate-biased (Wilson, Ruiz, & Davidowitz, 2019) and their host plants tend to be carbohydrate-biased relative to their IT (Wilson, Ruiz, Duarte, et al., 2019). However, this does not seem to translate to population level effects, potentially due to secondary metabolites affecting growth more strongly than macronutrient balance and/or larvae may be able to compensate by overeating carbohydrates to acquire sufficient protein (Wilson, Ruiz, Duarte, et al., 2019). Overeating carbohydrates is not as detrimental as overeating protein, at least in the short term, and animals tend to be willing to overeat carbohydrates to a greater extent than protein (Cheng et al., 2008; S. J. Simpson & Raubenheimer, 2012). Therefore, herbivores facing a nutritional landscape with a p:c generally lower than their IT (i.e., carbohydrate excess) may not be as negatively impacted as herbivores facing one higher than their IT (i.e., protein excess). However, there are several examples of higher localized densities of herbivores in response to higher plant nitrogen and protein contents with thrips (Brown et al., 2002) and spruce budworm (Choristoneura) (De Grandpré et al., 2022) being two examples. These examples suggest that low p:c diets limit population growth of some herbivores, but more studies are needed to determine if this relationship is only localized or if it scales up. It may be that herbivore populations with lower numbers are not limited by a nutritional landscape at a large scale because they can differentially disperse locally among optimal patches, whereas herbivore populations with extreme numbers (i.e., irruptions) may be more limited by nutritionally unfavorable environments across scales.

There is evidence for phosphorus limitation in some species, but limited research showing a detrimental effect of excess phosphorus (Cease et al., 2016). In aquatic insects such as Daphnia species, there is a strong positive association with phosphorus available and population dynamics (Andersen et al., 2004). However this trend is not seen in field cricket populations (Gryllus veletis) (Harrison et al., 2014) and other terrestrial insects. Loaiza et al., (2011) found no effect of phosphorus fertilization (but a positive effect of N fertilization) on Kansas tallgrass prairie grasshopper population distributions, whereas (Joern et al., 2012) found consistent positive correlations between plant phosphorus and Nebraskan grassland grasshopper populations.

Making predictions about a population’s nutritional demands can aid in making predictions about the relationship between nutritional landscapes and population dynamics. Across taxa, including fish, chickens, rats, cats, caribou, pigs, and dairy cattle, mass specific protein consumption is highly correlated with growth rate and decreases with age and body size (Talal, Harrison, et al., 2023). In contrast, energy demand (carbohydrates and lipids) does not show a clear relationship with growth rate and instead is more affected by environment and activity (Talal, Harrison, et al., 2023). Therefore, an animal’s IT is predicted to be affected by the contrasting effects of growth (increases dietary p:c) and activity or stress (increases carbohydrate demand and therefore decreases dietary p:c), although other physiological and environmental factors affect p:c demand as well (see Table 1 in Cease, 2024). For example, monarch butterflies have been gradually increasing their already-high daily energy expenditure during migration due to warmer temperatures caused by climate change (Parlin et al., 2023). Young and fast growing herbivores with low activity levels would be predicted to have a high p:c IT, whereas older juveniles and adults (slower mass specific growth) with high activity levels would be predicted to have a low p:c IT. Comparative studies with herbivores grouped functionally, such as other highly migratory animals (e.g. across insects, birds, mammals, and fish), or by growth rate or developmental stage, would likely provide interesting parallels that would assist in disentangling the complexities of plant macronutrient-herbivore relationships.

## 4.5 Synthesis and Application

Acquiring the right amount of nutrients is a critical component for animal growth, reproduction, and population dynamics (Doonan & Slade, 1995; Hansson, 1979; Keith, 1983). However, in contrast to the conventional hypotheses that predict a broad positive linear relationship between herbivorous populations and nitrogen and phosphorus (Huberty & Denno, 2006; Mattson Jr, 1980; White, 1978, 1993), the story is nuanced and probably most often non-linear. For some species, especially those with high energy requirements, the relationship is the opposite (negatively associated with nitrogen) like many locust species and the effects can be seen at the continental scale. Land use and Land Cover Change (LULCC) impact on nutritional environments has important implications for animal population dynamics from conservation to pest management. While we did not make an explicit connection between LULCC and locust outbreaks in Australia, our results are consistent with previous research showing that LULCC that decreases soil quality and creates low nitrogen environments increases physiological performance and outbreaks of locusts (reviewed in Le Gall, Overson, et al., 2019). Most importantly, we show that this relationship is consistent between scales from the individual locust to continental wide outbreaks. As such, proper management of soil nutrients can help keep locust populations from reaching outbreak sizes and should be considered across scales, from individual locust behavior to continental wide plagues.