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ORIGINAL RESEARCH

Stable Isotope Analysis of the Meat Ant (*Iridomyrmex purpureus*)

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ABSTRACT:

Stable isotope technology has greatly contributed to our understanding of the food web ecology of social insects, and can determine the dietary history of organisms. Furthermore, this method has been utilized successfully to study nutrient fluxes enriched with carbon and nitrogen isotopes. The aim of this study is to show if there is a relationship between δ13C and δ15N of meat ant nests and comparing the ration with soil adjacent to the ant nests. This study was carried out on 62 nests of meat ants (*Iridomyrmex purpureus*) and 62 samples of soil located adjacent to the nests in Armidale in New South Wales, Australia. The first ants nest was sampled at UNE, Armidale, and the following 61 nests were sampled in a northerly direction away from UNE on a wide road verge/ travelling stock route. There were no significant differences between the C and N stable isotopes collected from soil and ants nests and distance that nests were located away from one another; and there were no significant differences between δ13C and δ15N and the size of the ants nest (based on the N-S nest length). Finally, the δ15N levels of meat ants were not correlated with their surrounding soil.

Future study of measuring δ13C and δ15N levels to investigate particular interactions among ants and surrounding soil with other resources that ants may feed on will be necessary to observe ants in a wide range of environments and that it will increase our understanding of ants' food webs.

Keywords: Meat Ants (Iridomyrmex purpureus), Colony of Meat Ants, Stable Isotope.

INTRODUCTION

In Australia, the meat ant (*Iridomyrmex purpureus*) is a dominant ant in south eastern Australia (Andersen & Patel, 1994), and it is one of the 60 species of Iridomyrmex in Australia (Shattuck & Barnett, 2001). The meat ants'colony is polygynous (a mating pattern in which a single individual mates with more than one individual of the opposite sex) (Hölldobler & Carlin, 1985), but less than 20% of the galleries are controlled by queens (Greaves & Hughes, 1974). Hölldobler and Wilson (1990) suggested, however, that many mature nests consist of more than one functional (egg-laying) queen (Hölldobler & Carlin, 1985). Workers (sterile females) perform all other colony activity such as feeding the young, serving the queen, collecting water and food, and protecting the nest (Wilson, 1976). Workers are also genetically recognisable as solitary family units combined to form huge multi-nest colonies with a considerable exchange of workers among the nests (Halliday, 1983). Workers perform most jobs within the colony: some assist young queens to establish colonies and others attack strangers, indicating that they might be able to discriminate between outsiders and their nest mates (Carew, *et al*., 1997).

Ants can be herbivorous, carnivorous or omnivorous (Wootton, 1975), consuming a wide variety of plant material, invertebrates and vertebrates (Hölldobler & Wilson, 1990; Scheu, 2001), and they take their nutrition directly by trophobiosis from plants in a similar fashion to phloem-feeding insects (Stadler & Dixon, 2005). They also consume plants exudates, animal tissues, and honeydew produced by herbivorous insects and seeds (Carroll & Janzen, 1973; Hunter, 2009). There are some features of using stable isotope method: stable isotopic composition reflects some aspects of the organisms' diet such as the mean trophic level occupied by every species and the main sources of their energy (DeNiro & Epstein, 1978, 1981; Gannes *et al*., 1997; Ponsard & Arditi, 2000b).

Moreover, stable isotope analysis not only provides a way to limit the dietary history of organisms but it also provides an obvious way to understand the food web ecology of social insects (Tillberg,*et al*., 2006). (DeNiro & Epstein, 1978; Fisher, *et al*., 1990; Ostrom, *et al*., 1996; Ponsard & Arditi, 2000b Post, 2002 ; Hood - Nowotny & Knols, 2007; Nitrogen isotopes can be utilized to indicate the trophic level of consumers (Peterson & Fry, 1987;Cabana & Rasmussen, 1996;Post, 2002), with a common pattern of 3 to 4% enrichment with every trophic exchange (DeNiro & Epstein, 1981); additionally that means nitrogen enrichment comes at a much greater percentage than carbon enrichment (Ponsard & Arditi, 2000b ;Hood - Nowotny & Knols, 2007). Although stable isotope has limited uses for some applications of food webs of agro ecosystems with some exceptions (Ostrom *et al*., 1996 ;McNabb, *et al*., 2001;), it has been widely used to investigate many terrestrial ecosystems in both temperate and tropical areas (Ponsard & Arditi, 2000b ;Davidson,*et al*., 2003; Halaj,*et al*., 2005;). In recent years, Tillberg *et al*. (2006) suggested a standardized structure to the utilization of stable isotopes to measure the trophic ecology of ants (Ottonetti, *et al*.i, 2008). This study initially focused on 2 questions: First, how do the stable isotopes of meat ants change with distance away from each other?. Second, do ants have more variable Nitrogen (N) than Carbon (C) ratios compared to the environments in which they are found?.

MATERIALS AND METHODS

Background on Stable Isotopes

Stable isotopes have the benefit of logical variances in the percentages of heavy to light isotope in biological pertinent elements, for example, nitrogen and carbon, as they are expressed in (δ) notation as parts per thousand (0/00) as determined by

$$\delta X = \{(Rsample / Rstandard) - 1\} * 1000$$

Where X is 13C or 15N

Rsample and Rstandard mean the corresponding ratio (13C/ 12C) or (15N/ 14N). The carbon stable isotope ratios are expressed relative to the international Pee Dee Belminite (PDB), while the nitrogen stable isotope ratios are expressed relative to atmospheric nitrogen (AIR).

Due to several biological procedures such as ‗fractionation', a rise in the heavy light isotope ratio means enrichment, and a reduction in this percentage means depletion. The most commonly utilised isotope elements in environmental studies are carbon (13C) with a natural abundance at 1.108%, and nitrogen (15 N) at 0.3663% (Tillberg et al., 2006). For the meat ant samples, we used Δ X = and Δ 15N (%), as Δ represents the isotopic fractionation or trophic enrichment factor between dietary and consumer tissue.

Ant Samples Preparation

After collecting 62 samples of ant from 62 nest (n=62), and 62 samples of soil surrounding the nest (62), all samples were taken to the Insect Ecology Laboratory at the University of New England and stored at -15oC until processing. Ant thoraxes were utilized for isotope analysis to eliminate the effect on the isotopic analysis of undigested food in the abdomen, and cut off in a petri dish (50mm x 9mm). The thorax of the ant samples was weighed in a small cup on a micro-analytical balance, placed in small cylindrical tin capsules (8mm in height x 5mm in diameter), and put into 96 well cell culture cluster ready for analysis through isotope ratio mass spectrometry (IRMS); the weight of ants' thorax ranged from ~1 to 1.5 mg.

Soil Samples Preparation

All soil samples was taken 1m adjacent to each ant nest (n = 62). Before analysis, all soil sampling was oven-dried at 18o C for 24 hours. After drying, the samples were ground in a mortar and pestle until the particles were < 0.5 mm, then the soil samples were weighed in a small cup on a micro-analytical balance. The weight range of the soil sample was between 30 and 40 mg. The samples were then placed in small cylindrical tin capsules and put into 96 well cell culture clusters.

Data Analysis

The data were analysed by using Image J® to test for a relationship among nests. Distances were measured with Mapsource® , and a linear regression was used to compare relationships between Nest 1 and the other Nests in the R program (version 2.14.1); the graphs were designed with Scatter Plot in R. In order to determine whether the distance between nests, which means the distance that each ants nest is from nest ‗0' (UNE) measured using GPS coordinates, differed in δ13C and δ15N levels for ants and the soil, linear regression were used (n = 62). The graphs relating to the distance between ants nest were designed by Sigma Plot graphing software (version 12.2). To examine the relationship between nest lengths (North-South length measured using a tape measure), and nest area (ants nest area in m2), linear regression was used and the related graphs were designed using Scatter Plot in R.

To test the differences between δ13C and δ15N in the ants, nest areas and soil linear regression was used and the related graphs produced in Sigma Plot® 12.0.

RESULTS AND DISCUSSION

Meat Ant Isotope Values and Distances between Nests (Km)

There were no significant differences between the distance between nests (Km), which is the distance that each ants nest is from nest ‗0‘ (UNE) measured using GPS coordinates , and meat ants nest isotope values; δ13C and δ15N in ants Table (1).

Table:(1). Statistical Output of δ13C and δ15N Isotope Values in Meat Ant.

Isotope	Estimate(%)	Std. Error	T value	P value
Carbon	1.14	1.28	0.89	0.37
Nitrogen	1524.62	1330.43	1.15	0.25

Soil Isotope Values and Distance between Nests (Km)

The distance between nests (Km) and δ 13C values in soil surrounding the nest shown no significant difference; soil δ 13C (t = -1.124, P= 0.266).

However, there was significant difference between the distance between nests and δ 15N values and soil surrounding the nest: soil δ 15N (t =3.647, P < 0.001 Table (.2).

Table:(2). Statistical Table of δ^{13}C Soil and δ^{15}N Soil Isotope Values with Significant Values inbold.

Isotope	Estimate(%)	Std. Error	T value	P value
Carbon	-0.68	0.60	-1.12	0.27
Carbon	-0.68	0.60	-1.12	0.27

Overall, although there was a significant difference between the distance and δ13C and δ15N of the soil surrounding the nest, unlike the meat ant δ13C and δ15N values, the relationship between δ13C values in the soil surrounding the nest and δ13C values in meat ant and the distance were no significant Figure (1).

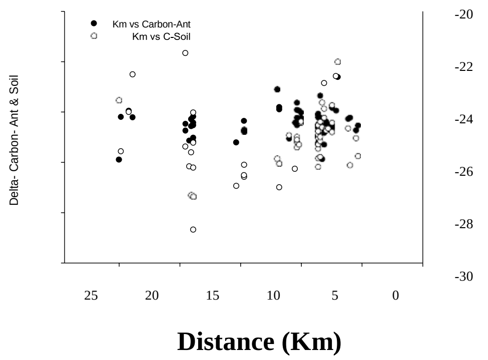

Figure: (1). δ13C {Delta-Carbon-Ant} (0/00)at different meat ant nests (n=62), and δ13C {Delta-Carbon-Soil} (0/00) from the surrounding soil in each nest (n=62) and the distance (Km) from Nest 1 (found at 0).

Moreover, the relationship between δ15N values in the soil surrounding the nest and δ15N values in meat ant and the distance was no significant Figure (2) ; R2 of the soil δ13C and δ15N ratios = 0.20, and of meat ant δ13C and δ15N ratios R2 = 0.049.

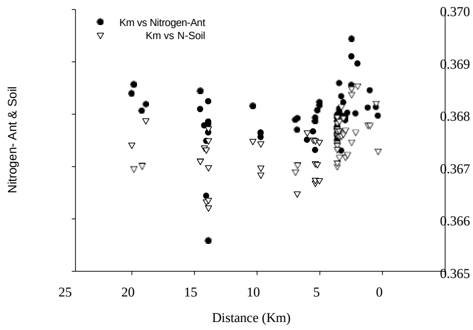

Figure: (2). δ^{15}N {Nitrogen-Ant} ($^0/_{00}$) at Different Meat Ants Nests (n=62) and δ^{15}N{Nitrogen-Soil} ($^0/_{00}$) from the Surrounding Soil (n=62) and the Distance (Km) from Nest1(found at 0).

Meat Ants and Soil Isotope Values in Relation to Nest Characteristics

The nest length (North-South): and carbon isotope values in meat ants and soil surrounding the nest shown no significant difference (Figure (3), where δ13C in meat ant: P = 0.53, and T- value = 0.63, and the soil surrounding the nest, where (P) δ13C values in soil = 0.50, and T- values = -0.68, also the relationship between the nest length and δ13C meat ant and δ13C in soil (R2 = 0.03), P = 0

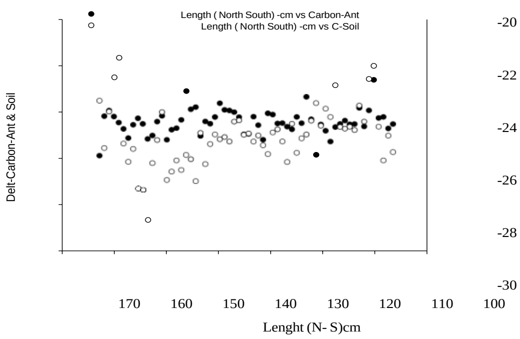

Figure: (3). δ15N {Nitrogen-Ant}(0/00at Different Meat Ant Nests (n = 62), and δ15N{Nitrogen-Soil} from the Surrounding Soil (n = 62) and North-South Aligned ant Nest Length (cm) : it is the length of the ants nest aligned N-S measured with a step measure at each nest.

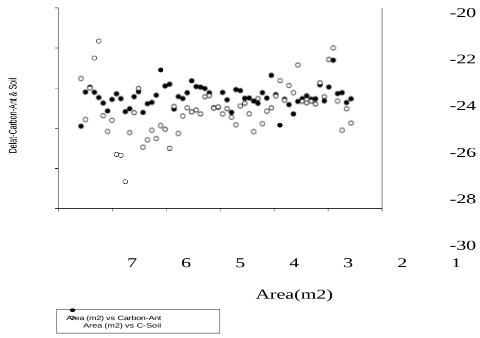

Figure: (4). δ13C {Delta-Carbon-Ant} (%) at different meat ants nests (n= 62) and δ13C

{Delta-Carbon-Soil} (0/00) from the soil surrounding the nest (n=62) and the nest area (m2).

Additionally, there was no significant difference between the nest area (m2) and nitrogen isotope values in meat ants and soil surrounding the nest (fig. 3.6), w here δ15N values in ant: P = 0.263, and T= -1.131, and δ15N in soil: P= 0.282, T= -1.087, and the relationship between the nitrogen isotope values in meat ants and soil surrounding the nest and the nest area (m2 R2 = 0.055)

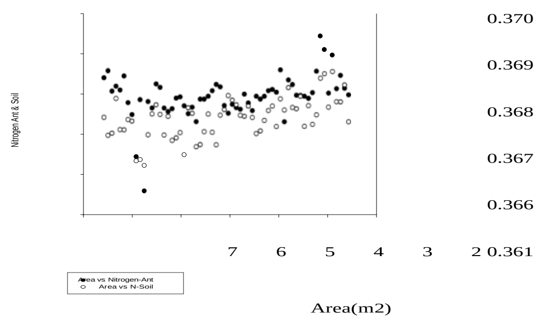

Figure: (5). δ15N {Nitrogen-Ant} (0/00) at Different Meat ant Nests (n=62) and δ15N {Nitrogen- Soil} (0/00) from the Soil Surrounding the Nest (n=62) and the Nest Area (m2).

The nest area (m2) and carbon isotope values in meat ants and soil surrounding the nest shown no significant difference (Fig. 3.5), where δ13C values in ant: P = 0.57, and T = 0.57, and δ13C values in soil: P = 0.46, T= -0.74, and the relationship between the carbon isotope values in meat ants and soil surrounding the nest and the nest area was not significant (R2=0.05).

The Relationship between Meat Ant Isotope Values and Soil Isotopes Values

Surprisingly, there were significant differences in the relationship between Carbon and Nitrogen from ant nests and the soil surrounding the nest (Figure 3.7; SMATR Test stat = 34.503, P = 0.001), with soil samples exhibiting a significant positive relationship (n = 66, R2= 0.364, p<0.0001) and ants also exhibiting a significant positive, but weak relationship (n = 63, R2 = 0.095, p=0.014).

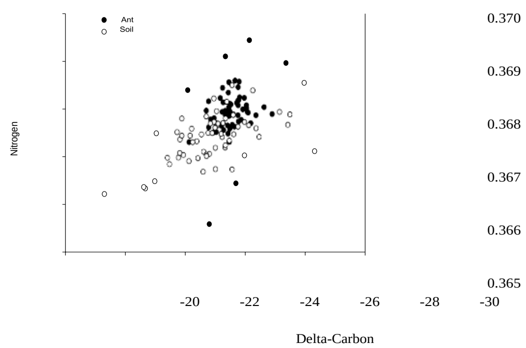

Figure: (6). The relationship between δ^{13} C {Delta-Carbon soil} (%) and δ^{15}N {Nitrogen- soil}($^{0}/_{00}$) isotope values from the Soil Surrounding the Nest (n=62) and δ^{13} C {Delta-Carbon- Ant}($^{0}/_{00}$) and δ^{15}N {Nitrogen-Ant}(%) Isotope Values of Ants at Different Nests (n=62)

Distances between nests and meat ant isotope values

The relationship between the distance that each ants nest is from nest ―0‖ (UNE), and nest site was not significantly different.

This result may be due to a number of reasons: (1) the distance, which was measured among the nests, was small (26 Km); therefore, the changes of carbon and nitrogen isotopic content would not be significant as they are similar types of habitat; (2) ants' nests are built in a homogenous environment, so this environment may have similar food resources; (3) the meat ants may have established their nests in similar site positions as well as closer to food resources to reduce the average time to travel for foraging.

This is shown in the positive relationship between the maximum distance between nests and the maximum distance between food resources (Van Wilgenburg & Elgar, 2007).

The Distance between ant Nests and Adjacent Soil Isotope Values

The relationship between the distance between nests and soil from surrounding soil (meat ant habitat) varied as the difference between δ15N in soil and the distance was significant (p< 0.001).

Whereas there was no significant difference between δ13C in soil and the distance (p = 0.27). My result could be inferred by a number of factors: soil δ15N values might be higher than soil δ13C values because of nitrogenous fertilisers.

In fact, during the 1970s and 1980s,, many studies mainly concentrated on tracing fertilizer nitrogen in intensive agriculture (Kohl, *et al*., 1971; Meints, *et al.*, 1975) and livestock movement.

Several studies have reported variations in 15N abundance in natural settings (Hoering, 1955; Pakwel *et al*., 1957; Wellman et al. 1968; Delwiche & Steyn, 1970). A study by Evans (2007) also found that variations between agriculturally managed and undisturbed sites were identified in δ15N soil.

The Relationship between Meat Ant and Soil Stable Isotopes

A previous study by Jung and Lee (2012) found that the variation of trophic position of ants may be due to highly variable feeding behaviours.

This result concurs with other studies that found there was a considerable variation in δ15N between colonies of the same species (Mooney & Tillberg, 2005). Another reason for δ15N meat ant variation would be differences in dietary history between the meat ant colonies (Tillberg *et al*., 2006).

This variation of δ15N in meat ants concurs with many other studies that indicated there was significant variation in Nitrogen isotope values through ant subfamilies and genera (Fiedler *et al*., 2007).

It also could be due to the regular uptake of mammal and bird faeces as well as arthropod corpses (Hahn & Maschwitz, 1985). Other studies have shown slightly high δ15N levels among species, and they explain that it may be because of ants feeding on plant food bodies (;Sagers *et al*., 2000; Fischer *et al*., 2002).

CONCLUSION

Stable isotope method proved to be a useful tool for assessing the trophic level of ants in the food web as they use a great diversity of food sources.

However, there were significant differences between the ants and the surrounding soil that may be explained by the different food resources the ants use.

Stable isotope technique led to a classification of the ant species as herbivorous; they have higher δ13C because they likely feed on nectar or honeydew or they are omnivores; if they have higher δ15 N they likely feed on arthropod corpse and mammal and bird faeces, which was previously demonstrated by measuring the level of δ13C and δ15 N.

Future study on assessing trophic level of ants will provide a refined picture of ants‘food webs but it should be done over a wide range of environments.

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الملخص

ساهمت تقنية النظائر المستقرة بشكل كبير في فهمنا لبيئة الشبكة الغذائية للحشرات الاجتماعية، ويمكنها تحديد التاريخ الغذائي للكائنات الحية. علاوة على ذلك، استُخدمت هذه الطريقة بنجاح لدراسة تدفقات المغذيات المُخصبة بنظائر الكربون والنيتروجين. تهدف هذه الدراسة إلى إثبات وجود علاقة بين δ13C وδ15N في أعشاش نمل اللحم، ومقارنة هذه النسبة مع التربة المجاورة للأعشاش. أُجريت هذه الدراسة على 62 عشًا من نمل اللحم (Iridomyrmex purpureus) و62 عينة من التربة المجاورة للأعشاش في أرميدال، نيو ساوث ويلز، أستراليا. جُمعت عينة العش الأول في جامعة نيو إنجلاند (UNE) في أرميدال، بينما جُمعت عينات الأعشاش الـ 61 التالية باتجاه الشمال بعيدًا عن الجامعة، على جانب طريق واسع/ممر للماشية. لم تُلاحظ فروقٌ جوهرية بين النظائر المستقرة للكربون والنيتروجين المُستخلصة من التربة وأعشاش النمل، ولا بين المسافة بين الأعشاش؛ كما لم تُلاحظ

فروقٌ جوهرية بين δ13C وδ15N وحجم عش النمل (بناءً على طول العش من الشمال إلى الجنوب). وأخيرًا، لم تكن مستويات δ15N في نمل اللحم مرتبطةً بالتربة المحيطة به. لذا، ستكون هناك حاجةٌ لدراسةٍ مستقبليةٍ لقياس مستويات δ13C وδ15N للتحقق من التفاعلات الخاصة بين النمل والتربة المحيطة به، بالإضافة إلى الموارد الأخرى التي قد يتغذى عليها النمل، وذلك لمراقبة النمل في بيئاتٍ متنوعة، مما سيزيد من فهمنا لشبكات الغذاء لدى النمل.

الكلمات المفتاحية: نمل اللحم (Iridomyrmex purpureus)، مستعمرة من نمل اللحم، نظير مستقر.