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Stable isotope analysis to assess the trophic level of arthropod in sugarcane ratoon agroecosystem

HERI PRABOWO[1,2], BAMBANG TRI RAHARDJO[1,♥], GATOT MUDJIONO[1], AKHMAD RIZALI[1]
[1]Department of Plant Pest and Diseases, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia.
Tel.: +62-341-51665, 565845, Fax.: +62-341-560011, ♥email: bambangtri@ub.ac.id
[2]Indonesian Sweetener and Fiber Crops Research Institute. Jl. Raya Karangploso Km. 4, Malang 65152, East Java, Indonesia

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Abstract. *Prabowo H, Rahardjo BT, Mudjiono G, Rizali A. 2022. Stable isotope analysis to assess the trophic level of arthropod in sugarcane ratoon agroecosystem. Biodiversitas 23: 2871-2881.* Arthropods represent one of the main components of soil inhabitants and play an important role in maintaining soil health, as well as providing ecosystem services. The description of the trophi c level of the ratoon sugarcane agroecosystem is needed to describe the role of organisms in the ecosystem to maximize the role of detritivores, predators, and parasitoids in the ratoon sugarcane agroecosystem. The stable isotope approach is widely used in various studies to describe trophic levels in an agroecosystem. The stable isotope technique, especially the one that uses stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), can measure the trophic position that integrates energy assimilation or mass flow through all the different trophic pathways leading to an organism. Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ can be used to identify the roles of arthropods in the ratoon sugarcane agroecosystem by identifying the composition of both isotopes. The ratio of arthropod's carbon assimilation ($\delta^{13}C$) to sugarcane ranges from-1.4 to-5.45‰. In contrast, the ratio of nitrogen assimilation ($\delta^{15}N$) of arthropod to sugarcane ranges from 3.86 to 39.7‰. The values of stable isotope $\delta^{13}C$ and $\delta^{15}N$ on predator and parasitoids are varied. The stable isotope value of carbon ($\delta^{13}C$) for predators varies from-10.14 to-11.62‰. In contrast, the predator's stable isotope value of nitrogen ($\delta^{15}N$) varies from 9.17 to 18.1%. The parasitoids' carbon stable isotope value ($\delta^{13}C$) varies from 10.5 to 11.05‰. In contrast, parasitoids' nitrogen stable isotope value ($\delta^{15}N$) varies from 12.8 to 17.05‰. The value of carbon (δ13C) stable isotope assimilation between herbivores and predators varies from 0.006 to 1.38‰. While the value of nitrogen ($\delta^{15}N$) stable isotope assimilation varies in the range of 0.33 to 10.3‰. Furthermore, the value of carbon ($\delta^{13}C$) stable isotope assimilation between herbivores and parasitoids varies in the range of 5.3 to 9.23‰. While the value of nitrogen ($\delta^{15}N$) stable isotope assimilation varies in the range of 3.79 to 10.3‰. Isotope content ($\delta^{13}C$) shows the food resources of arthropods in the agroecosystem, while isotope value ($\delta^{15}N$) shows the roles of arthropods in the sugarcane ratoon agroecosystem. Carbon stable isotope values of predator and parasitoids are close to zero. While the stable nitrogen isotope ($\delta^{15}N$) values on arthropods are averagely above 10‰, values are suspected of having roles as predators or parasitoids. Knowing the trophic level of predators and parasitoids through stable isotopes in agroecosystems can be used to conserve and optimize natural enemies to suppress the development of herbivores.

Keywords: Assimilation, parasitoid, predator, sugarcane

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the important industrial crops grown globally (Prabowo et al. 2021). Sugarcane farming increases income as well as a source of important energy material that can promote sustainable development (Kaab et al. 2019; Gonçalves et al. 2021). One key factor in increasing sustainable sugarcane productivity is soil health management support (Moebius-Clune et al. 2016; Menta and Remelli 2020). Organic matter combined with nutrient resources, such as animal manures, crop residues, and green manuring, is increasingly used to replenish organic matter and improve soil structure and fertility. A growing body of research indicates that organic farming results in higher soil quality and more biological activity in soil than conventional farming. Organic farming methods have also been shown to use nutrients and energy more efficiently than conventional farming methods (Singh et al. 2005). The practice that is currently developing to maintain soil health is by implementing organic material management of soil to preserve the abundance and diversity of micro and macro-organisms, which will finally work in synergy to support plant health (Nanganoa et al. 2019; Selim 2020; Sulok et al. 2020)

Arthropods represent one of the main components of soil inhabitants and play an important role in maintaining soil health, as well as providing ecosystem services. Soil arthropods are involved in many processes, such as translocation of organic materials, breakdown and decomposition, nutrient cycle, formation of soil structure, and water regulation. Besides, several groups are highly sensitive to changes in soil quality because they live, eat, and breed in the soil and are extremely adaptable to specific soil conditions. Among the soil microarthropods, Collembola and Acari are the two most important groups of abundance and diversity and are the most widely studied taxa to be developed as bioindicators (Suheriyanto et al. 2019). Both groups are often studied at the family, genus, or species level, and different non-taxonomy approaches consider functional groups or functional characteristics (Bagyaraj et al. 2016). Other microarthropod taxa that are often used to determine soil quality are (i) insects, like larva and adult Coleoptera, Hymenoptera (especially ants), Diptera larva, (ii) Araneae, and (iii) Isopoda. However, other groups such as Protura, Diplura, Pseudoscorpionida, Symphyla, and Pauropoda are often used as supporting data for soil health bioindicator parameters other than Collembola and Acarina (Moretti et al. 2017; Galli et al. 2019).

Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios are used to investigate the trophic interactions of species in situ, which is particularly beneficial for researching cryptic systems such as those found beneath the ground. The technique was pioneered in ecology more than three decades ago. During the first decade, it was primarily used to study aquatic systems (William and Garman 1996; Perkins et al. 2014; Guiry 2019; Krause et al. 2019). Some early studies recognized the method's advantages for studying soil animals; however, these studies were focused on large and relatively well-studied taxa, such as earthworms and four termites (Kupfer et al. 2006; Hyodo et al. 2008). Natural variations in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios are increasingly being used to characterize soil animal trophic niches and provide insight into trophic levels, basal resources, and the trophic structure of entire communities (Potapov et al. 2019). Animal tissue is enriched in ^{15}N by about 3.4h per trophic level, allowing researchers to study how species' trophic positions change in response to environmental changes. Unlike ^{15}N, ^{13}C is little enriched in consumers compared to their diet, allowing for identifying basal food resources in food webs. Stable isotopes have been used to investigate the trophic niches of various soil invertebrates. However, they have rarely been used to investigate how changes in soil organism trophic niches are affected by changes in land use. Furthermore, previous studies based on stable isotopes did not consider the abundance and/or biomass of the studied species, implying that all species were assumed to be of equal importance or impact. These deficiencies can be accounted for using novel techniques yet to be applied to soil communities (Layman et al. 2012; Qin et al. 2021; Alp and Cucherousset 2022).

Stable isotopes have the potential to capture complex interactions, such as trophic omnivory, while also tracking energy or mass flow through ecological communities. Because a consumer's diet is typically enriched by 3-4 relative to its diet, the ratio of stable isotopes of nitrogen ($\delta^{15}N$) can be used to estimate trophic position. In contrast, the carbon isotope ratio ($\delta^{13}C$) changes little as carbon moves through food webs and, as a result, can typically be used to evaluate the ultimate sources of carbon for an organism when the isotopic signatures of the sources differ (Page et al. 2013; Perkins et al. 2014; Villamarín et al. 2018)

Arthropods play an important role in the ratoon sugarcane agroecosystem due to their numerous benefits. The position of the tropic level in the agroecosystem must be known to maximize its role. A description of the tropic level of the ratoon sugarcane agroecosystem is required to maximize the role of detritivores, predators, and parasitoids in the ratoon sugarcane agroecosystem. The stable isotope approach is widely used in various studies to describe trophic levels in an agroecosystem. The stable isotope technique, especially the one that uses stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), can measure the trophic position that integrates energy assimilation or mass flow through all the different trophic pathways leading to an organism.

MATERIALS AND METHODS

Research location and time

This research was conducted from February 2021 to December 2021. Arthropods for isotope analysis were collected from a sugarcane ratoon plantation in Karangploso Experimental Station, Indonesian Sweetener and Fiber Crops Research Institute, Karangploso Sub-district, Malang District, Indonesia, at the altitude of the 515 m above sea level (a.s.l.), climate type C3 (Based on the Schmidt-Ferguson Classification). It has an inceptisol soil type with a sandy loam soil texture. It is located at 7°54'28"S, 112°37'30"E. The research was also conducted in the Laboratory of Entomology and Phytopathology at the Indonesian Sweetener and Fiber Crops Research Institute of Malang, especially for the clarification and identification processes of the results of arthropods based on the morphospecies. In addition, an analysis of stable carbon isotope ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values was conducted in the National Nuclear Energy Agency of Indonesia, Jakarta as well as Hydrogeology and Geochemistry Laboratory, Bandung Institute of Technology, Bandung, Indonesia.

Arthropods were trapped with modified Berlese-Tullgren Funnel. The collected arthropods were then filtered and washed with deionized water. Arthropod preparation followed the modified method of Saccò et al. (2019). Arthropods were sorted based on taxa using a stereo microscope. The arthropods obtained were identified at least at the family/morpho-species level based on the insect identification book written by Kalshoven (1981), Capinera (2008), Hill (2008), Gullant and Cranston (2010), and Emden (2013), Farrow (2016). The arthropod was finely grounded until the fresh weight of arthropods was as much as 10 grams. The sample was then dried in a desiccator for 7 days. The dried sample was prepared for isotope analysis. The sample was analyzed for 30 arthropod types and chosen by their roles in the ecosystem (Table 1). The sample was analyzed 3 times of repetition.

Stable isotope preparation and analysis used an approach through two elements of carbon and nitrogen, which were $\delta^{13}C$ and $\delta^{15}N$. These two elements are chosen because they are commonly used to describe the food chain in the ecosystem. Isotope analysis used the mass spectrometer tool (Isotope Ratio Mass Spectrometry (IRMS), Finnigan Delta Plus and FlashEA 112 series, Thermo Fisher Scientific, Waltham, MA, USA), which is connected to elements of analysis (NA-2500, CE Instruments) with correction presentation of 0.15% conducted at the National Nuclear Energy Agency of Indonesia, Jakarta and the Hydrogeology and Geochemistry Laboratory, and Bandung Institute of

Technology. Value of stable isotope ratio used the conventional standard (*Pee Dee Belemnite*, PDB) of limestone for carbon and atmospheric N2 for nitrogen) (Hoefs 2009) by the formula $\delta^{13}C$ and $\delta^{15}N$ = (*Rsample*/*Rstandard* – 1) 1000 (‰). In which *Rsample* is the element of 13C or 15N, while *Rstandard* is the ratio of 12C or 14C based on PDB. Carbon standard of $\delta^{13}C$ used PDB, while nitrogen $\delta^{15}N$ used atmospheric N_2 gas standard. To calculate food sources assimilated by natural enemies (ratio $\delta^{13}C$ or $\delta^{15}N$), the formula (DeNiro and Epstein 1978, 1981): ΔAnimal-Diet was applied. Where Δ is the assimilation value of $\delta^{13}C$ or $\delta^{15}N$.

Data analysis

Statistic descriptive was applied to identify the average value of $\delta^{13}C$ and $\delta^{15}N$ of each sample. Besides, the data normality test was used for each $\delta^{13}C$ and $\delta^{15}N$ value of each sample using Kolmogorov-Smirnov test. To differentiate $\delta^{13}C$ and $\delta^{15}N$ value from arthropods and food sources, it was analyzed using ANOVA with program R version 3.6.0.

RESULTS AND DISCUSSION

Primary production of carbon and nitrogen isotopes and arthropods in ratoon sugarcane agroecosystem

Soil arthropods in the ratoon sugarcane agroecosystem have various roles, some are beneficial while others are detrimental. Description of arthropods' role in agroecosystem is needed to optimize its role, especially the role as natural enemy and decomposer; hence it is expected to be able to optimize ecosystem services to increase ratoon sugarcane productivity. In the developing study, a stable isotope approach is widely used to identify the roles of arthropods in tritrophic interactions. Implementation of this method can be used to point out the ecology and biological processes that occur in the ecosystem, especially the utilization process of food resources, tritrophic interactions, arthropods dispersal, predation, herbivore attack, etc. (Hood-Nowotny and Knols 2007).

Based on the analysis of variance (ANOVA), the value between carbon ($\delta^{13}C$) ($F_{29.60}$=3.72, P=0.000001) and nitrogen (δ15N) ($F_{29.60}$=27.74 P=0.000001) of arthropods found with the potential food resources have a significant difference. As the primary producer in the ratoon sugarcane agroecosystem, sugarcane has various average carbon and nitrogen isotopes (Table 2). The average values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are-12.26‰ and 4.29‰, respectively (Figure 1).

Table 1. Organisms used for the description of tritrophic interactions in the ratoon sugarcane agroecosystem

Organism	Role in agroecosystem	Phyllum/Division	Class	Order	Family
Sugarcane (PS 862)	Autotrof	Spermatophyta	Liliopsida	Poales	Poacea
Brachystomella	Detritivore	Arthropod	Collembola	Poduromorpha	Neaurinidae
Vitronura	Detritivore	Arthropod	Collembola	Poduromorpha	Neaurinidae
Alloscopus	Detritivore	Arthropod	Collembola	Entomobryomorpha	Emntompbryidae
Trombidium	Detritivore	Arthropod	Arachnida	Trombidiformes	Trombidiidae
Hypoaspis	Predatore	Arthropod	Arachnida	Mesostigmata	Laelapidae
Parcoblatta	Detritivore	Arthropod	Insect	Blattaria	Ectobiidae
Aleurolobus	Herbivore	Arthropod	Insect	Hemiptera	Aleyrodidae
Lepidiota	Herbivore	Arthropod	Insect	Coleoptera	Scarabaeidae
Pericalus	Predatore	Arthropod	Insect	Coleoptera	Carabidae
Chilo sacchariphagus	Herbivore	Arthropod	Insect	Lepidoptera	Crambidae
Scirpophaga excerptalis	Herbivore	Arthropod	Insect	Lepidoptera	Crambidae
Exypnus	Predatore	Arthropod	Insect	Dermaptera	Chelisochidae
Aulacaspis	Herbivore	Arthropod	Insect	Hemiptera	Diaspididae
Saccharicoccus	Herbivore	Arthropod	Insect	Hemiptera	Pseudococcidae
Oligonychus	Herbivore	Arthropod	Acarina	Trombidiformes	Tetranychidae
Ceratovacuna	Herbivore	Arthropod	Insect	Hemiptera	Aphididae
Phyllophaga	Herbivore	Arthropod	Insect	Coleoptera	Scarabaeidae
Lycosa	Predatore	Arthropod	Aranea	Aranea	Lycosidae
Tetragnatha	Predatore	Arthropod	Aranea	Aranea	Tetragnathidae
Dolychoderus	Predatore	Arthropod	Insect	Hymenoptera	Formicidae
Componothus	Predatore	Arthropod	Insect	Hymenoptera	Formicidae
Oecophylla	Predatore	Arthropod	Insect	Hymenoptera	Formicidae
Anax	Predatore	Arthropod	Insect	Odonata	Aeshnidae
Scolia	Parasitoid	Arthropod	Insect	Hymenoptera	Scoliidae
Telenomus	Parasitoid	Arthropod	Insect	Hymenoptera	Scelionidae
Isotima	Parasitoid	Arthropod	Insect	Hymenoptera	Ichneumonidae
Chalybion	Parasitoid	Arthropod	Insect	Hymenoptera	Sphecidae
Trichogramma	Parasitoid	Arthropod	Insect	Hymenoptera	Trichogrammatida
Cotesia	Parasitoid	Arthropod	Insect	Hymenoptera	Braconidae

Carbon value in the sugarcane leaves of the PS 862 variety is within the range of a previous study that agrees that the carbon isotope ($\delta^{13}C$) value in sugarcane ranges between-12.26‰ to 16.00‰ (Spain and le Feuvre 1997; Martinelli et al. 2002). Meanwhile, the nitrogen isotope ($\delta^{15}N$) value ranges between 3.2‰ to 5.00‰ (Spain and le Feuvre 1997; Ferger et al. 2013). Organic materials of sugarcane contribute to the amount of carbon in the agroecosystem, it covers the carbon contribution in air and soil. The return of organic matter to the land plays an important role in restoring carbon and organic matter in the land to maintain the sustainability of sugarcane productivity (Borges et al. 2018). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios are common organic matter flow and storage indicators inland research. The quantification and understanding of (i) the variability of isotope signatures of potential organic matter source materials; and (ii) the influence of organic matter decomposition on isotopic signatures are required to use these indicators effectively. While it is well known that organic matter properties change during decomposition, there has been little direct quantification of any concurrent shifts in isotope signatures for land detritus. The use of $\delta^{13}C$ and/or $\delta^{15}N$ inland studies to demonstrate and/or quantify the stability of carbon down sediment cores is becoming more common (e.g., Adame et al. 2019). Based on the predictive power of some Kelleway models, it is possible to use $\delta^{13}C$ and/or $\delta^{15}N$ as a quantitative indicator of decomposition status. However, capacity will vary between species and isotopes. More research into the relationships between decomposition and isotopic fractionation is needed for a broader range of species. Belowground tissues require special attention because they contribute significantly to long-term blue carbon storage (Donato et al. 2011) and respond differently than aboveground tissues of the same species. More quantification of early versus late-stage decomposition effects is also required to bridge the temporal gap between most decomposition studies (days to years) and blue carbon sequestration (decades to millennia) (Kelleway et al. 2022).

The stable isotope value of carbon and nitrogen can vary due to various factors. Isotope value is probably determined by physical factors such as shade, habitat, light, and temperature (Kiswara et al. 2005). Grice et al. (1996) believe that light influences the $\delta^{13}C$ value. This is due to the increase of $\delta^{13}C$ from external C sources and the increased recycling of CO_2. Carbon value in leaves is an overview of the resources of carbon, sunlight, and temperature (Hemminga and Mateo 1996).

Arthropods in the ratoon sugarcane agroecosystem play roles in levels two and three of tritrophic interactions. They mostly play the roles of herbivores, decomposers, and natural enemies. Results of the study prove that arthropods in the agroecosystem of ratoon sugarcane have various average carbon and nitrogen isotopes (Table 3). The average of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is-10.14 to-11.92‰ and 5.93‰ to 18.43‰, respectively. This range is within the range of carbon isotope value ($\delta^{13}C$) of arthropods of-29.00‰ and 4‰ (Coleman and Odum, 2015; Sabadel et al. 2019; Hernández-Castellano et al. 2021). Nevertheless, Rozanova et al. (2022) research believed that the carbon value ($\delta^{13}C$) of arthropods only ranged between 1.8 to 4.00‰. While the value range for nitrogen isotope ($\delta^{15}N$) of arthropods is still at the range of 1.8 to 16.00‰ (Schmidt et al. 2007; Birkhofer et al. 2016).

The difference in carbon and nitrogen isotope values in arthropods is influenced by various factors, which are environment, sunlight, food resources, evaporation, soil nutrient content, type of land use, and roles in an agroecosystem (Craine et al. 2015; van der Sleen et al. 2017; Susanti et al. 2021).

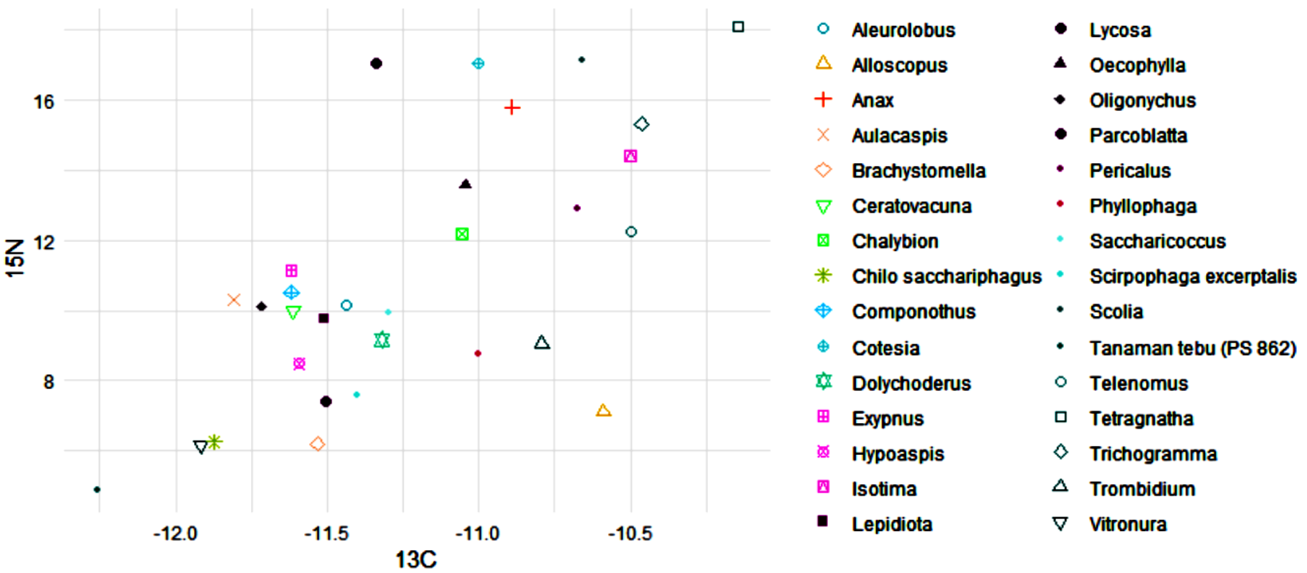

Figure 1. The value of the stable isotope ratio of arthropods in the ratoon sugarcane ecosystem

Table 2. Average value of (SD) $\delta^{15}N$ and $\delta^{13}C$ (‰) in ratoon sugarcane

Organism	$\delta^{13}C$		δ15N	
	Average (‰)	Range (‰)	Average (‰)	Range (‰)
Sugarcane of PS 862 variety	-12.26	-11.97 to-12.35	4.29	3.01 to 6.03

Table 3. Average value of (SD) $\delta^{15}N$ and $\delta^{13}C$ (‰) of arthropods in ratoon sugarcane field

Organism	δ13C		δ15N	
	Average (‰)	Range (‰)	Average (‰)	Range (‰)
Brachystomella	-11.53	-11.97 to-12.35	6.35	5.88 to 6.48
Vitronura	-11.92	-11.4 to-11.6	5.95	5.78 to 6.56
Alloscopus	-10.59	-11.87 to-11.99	7.22	5.78 to 8.86
Trombidium	-10.79	-10.11 to-11.44	8.99	9.2 to 9.2
Hypoaspis	-11.59	-10.2 to-11.87	8.91	7.64 to 9.14
Parcoblatta	-11.51	-11.55 to-11.66	7.33	6.89 to 7.77
Aleurolobus	-11.45	-11.21 to-11.8	10.02	9.54 to 10.5
Lepidiota	-11.51	-11.21 to-11.66	9.75	9.38 to 10.12
Pericalus	-10.68	-9.96 to-11.21	13.36	11.52 to 15.2
Chilo sacchariphagus	-11.87	-11.3 to-12.66	6.16	5.77 to 6.55
Scirpophaga excerptalis	-11.40	-11.02 to-11.66	7.79	7.12 to 8.19
Exypnus	-11.62	-11.34 to-11.9	11.27	10.9 to 11.44
Aulacaspis	-11.81	-11.58 to-11.97	10.34	10.24 to 10.34
Saccharicoccus	-11.29	-11.1 to-11.58	10.10	9.56 to 10.1
Oligonychus	-11.72	-11.3 to-11.97	10.20	9.99 to 10.5
Ceratovacuna	-11.61	-11.33 to-11.84	9.82	9.54 to 10.32
Phyllophaga	-11.00	-11.21 to-11.43	9.06	8.11 to 9.3
Lycosa	-11.34	-10.96 to-11.78	17.06	16.11 to 18.02
Tetragnatha	-10.14	-10.09 to-10.23	18.43	16.97 to 19.89
Dolychoderus	-11.32	-11.06 to-11.67	9.71	8.09 to 10.31
Componothus	-11.62	-11.32 to-12.22	10.68	10.22 to 11.04
Oecophylla	-11.04	-10.79 to-11.23	12.28	9.68 to 16.04
Anax	-10.89	-10.52 to-11.11	15.65	15.09 to 16.14
Scolia	-10.66	-9.66 to-11.66	16.39	15.24 to 16.2
Telenomus	-10.50	-10.1 to-11.2	13.29	10.22 to 18.64
Isotime	-10.50	-10.3 to-10.9	14.55	13.24 to 15.22
Chalybion	-11.06	-10.9 to-11.37	13.16	10.22 to14.1
Trichogramma	-10.46	-10.22 to-10.37	15.32	15.09 to 15.55
Cotesia	-11.00	-10.44 to-11.69	16.52	16.02 to 18.11

Stable isotope of arthropods and food resources in ratoon sugarcane agroecosystem

The ratio of arthropod's carbon assimilation ($\delta^{13}C$) to sugarcane ranges from-1.4 to-5.45‰ (Figure 3). In contrast, the ratio of nitrogen assimilation ($\delta^{15}N$) of arthropod to sugarcane ranges from 3.86 to 39.7‰ (Figure 2). The use of carbon and nitrogen isotopes can describe the interaction among food resources with tritrophic levels two and three.

Isotopic discrimination (also known as trophic shift or enrichment, is the difference between a consumer's and its prey's isotopic ratios resulting from the selective assimilation of heavy to light isotopes from consumed resources (McCutchan et al. 2003). Researchers must account for diet-tissue trophic discrimination factors before investigating nutrient flows, species interactions, trophic relations, or animal diets because they vary across species, tissues within species, and diets. Some invertebrates have had discrimination factors determined experimentally, but most insects do not. The identification of discrimination factors should be validated experimentally through controlled feeding trials; however, this is not always possible. The assimilation between the two causes a change in the value of carbon and nitrogen isotopes on measured arthropods (Haubert et al. 2005; Wolf et al. 2009; Quinby et al. 2020).

The assimilation value of arthropods on food resources shows different results depending on the type of arthropods and its role in the ratoon sugarcane agroecosystem. This assimilation value shows that the origin of food resources is used as energy resources to support the life of arthropods. Morphospecies with assimilation values close to the producer are *Vitronura, C. sacchariphagus*, *Brachystomella, Alloscopus,* and *Parcoblatta*; each assimilation value is 2.86, 4.17, 4.28, 6.72, and 7.64‰, respectively (Figure 3). The value of carbon assimilation ratio of food sources by consumers at the tritrophic level above it is in the range of-2 to +2 (Bouillon et al. 2008). While the value of nitrogen assimilation of food resources by consumers usually ranges from-0.7 to +9.2‰ (McCutchan et al. 2003). The difference in food resource assimilation is influenced by morphospecies, the physical condition of the microhabitat, environment condition, body size, predation, season, and roles in the ecosystem (Aya and Kudo 2010).

Stable isotope of predators and parasitoid on herbivores in ratoon sugarcane agroecosystem

Predators and parasitoids have vital roles in the ratoon sugarcane agroecosystem. Their existence is expected to suppress the herbivore population so that it does not cause economic loss for the ratoon sugarcane business. Therefore, a description of their interaction with herbivores in the ratoon sugarcane ecosystem is essential to optimize their roles. Based on the analysis of variance (ANOVA), the value of carbon (δ13C) ($F_{29.60}$=3.72, P=0.000001) and nitrogen (δ15N) ($F_{29.60}$=27.74 P=<0.0001) from arthropods found with food resources potential has a significant difference. The values of stable isotope $\delta^{13}C$ and $\delta^{15}N$ on predator and parasitoids are varied. The stable isotope value of carbon ($\delta^{13}C$) for predators varies from-10.14 to-11.62‰. In contrast, the stable isotope value of nitrogen ($\delta^{15}N$) for predators varies from 9.17 to 18.1‰ (Figure 1).

Carbon isotope values ($\delta^{13}C$) for predators indicate commonly consumed food resources. Carbon isotopes can describe predators' position in the agroecosystem by tracking the flow of nutrients and creating food webs. The stable isotope value of carbon reflects several aspects of predators' food with the approach of the main energy source for life support. In most of their life, predators meet the carbon element from predation on herbivores in the agroecosystem. The carbon ($\delta^{13}C$) isotope value for a predator is close to zero, which proves that predators do not eat directly from sugarcane plants in their life (autotroph). Arthropods with a predator role have a stable isotope value close to zero compared to autotroph plants. Spider predator, *Crematogaster scutellaris*, and *Lasius lasioides* have respective values by-25;-25; and-24‰. While the preys, such as aphids, grasshoppers, *Prays oleae, C. aethiops*, and Olive (*Olea europea)* have values of-29;-25;-24;-24, and-30‰, respectively (Ottonetti et al. 2008).

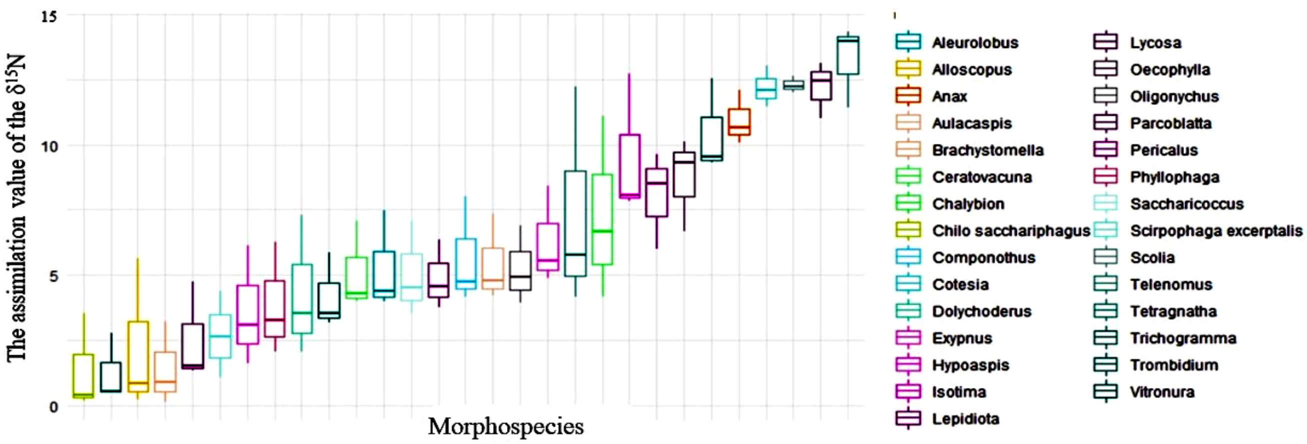

Figure 2. The assimilation value of the $\delta^{15}N$ ratio of arthropods with food sources in the ratoon sugarcane ecosystem (boxplot with different colors showing the type of morphospecies on the x-axis)

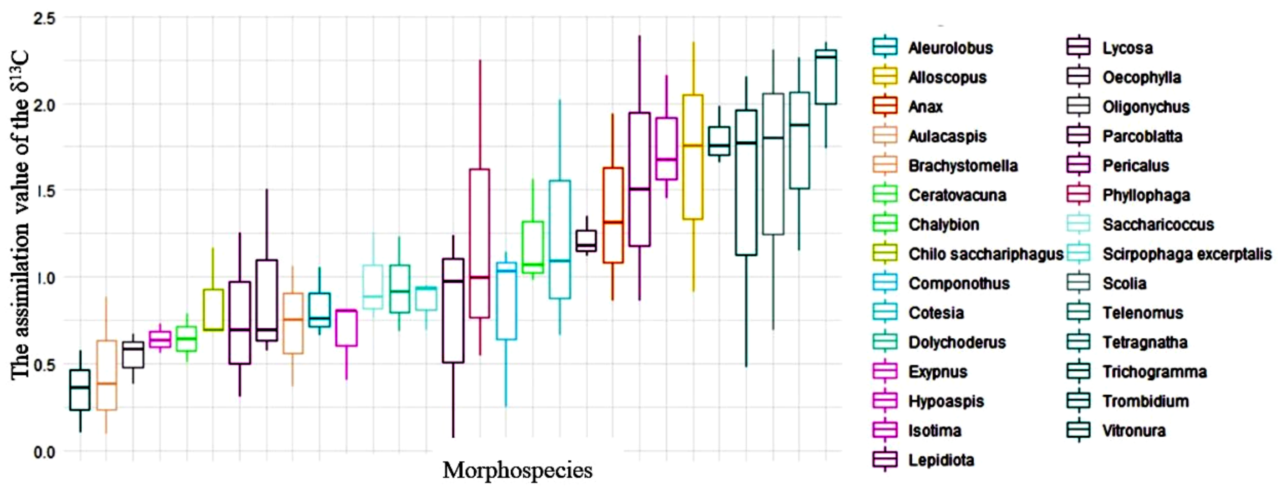

Figure 3. The assimilation value of the $\delta^{13}C$ ratio of arthropods with food sources in the ratoon sugarcane ecosystem (boxplot with different colors showing the type of morphospecies on the x-axis)

Besides stable carbon isotope, the role of a predator can also be seen from the nitrogen stable isotope value ($\delta^{15}N$). In their life, predators mostly eat arthropods in tritrophic level-two; hence, the accumulation of nitrogen content is relatively abundant compared to herbivores or autotrophs. As a result, nitrogen stable isotope values on arthropods are averagely above 10‰. Some studies also agree that arthropods as predators have higher nitrogen stable isotope value (δ15N) than herbivores. For instance, spider predator, *C. scutellaris* and *L. lasioides* that have values of 3.7; 3.3; and 3.6 ‰, respectively. While the preys, such as aphids, grasshoppers, *P. oleae,* and *C. aethiops* have lower values, which is by 1.6; 0.3; 0.3; and 1.9 ‰, respectively (Ottonetti et al. 2008). The order of highest nitrogen stable isotope values in the ecosystem is necrovores, predator, detritivores, and herbivores, which values are by 6.2,-2, and-4 ‰, respectively (Oelbermann and Scheu 2010).

The role of predators in the ratoon sugarcane agroecosystem is also shown by the stable isotope assimilation value of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) between predators and herbivores. Based on analysis of variance (ANOVA), carbon assimilation ($\delta^{13}C$) value ($F_{55.112}$=4.00, P=0.000001) and nitrogen ($\delta^{15}N$) ($F_{55.112}$=168.00, P=0.000001) from predator to herbivores have a significant difference. The value of carbon ($\delta^{13}C$) stable isotope assimilation varies from 0.006 to 1.38‰ (Figure 4). While the value of nitrogen ($\delta^{15}N$) stable isotope assimilation varies in the range of 0.33 to 10.3‰ (Figure 5). The variety of assimilation values is in line with other studies, which state that the values of carbon stable isotope assimilation ($\delta^{13}C$) on predators range between 4 to 5‰ (Ottonetti et al. 2008). The highest nitrogen assimilation value is obtained from the predation relationship between *Lycosa* and *C. sacchariphagus*. This agrees with studies conducted by Ottonetti et al. (2008) and Schmidt et al. (2007), which believe that nitrogen ($\delta^{15}N$) stable isotope assimilation on predators ranges between 0.3 to 2.1‰. The highest assimilation value is obtained on spiders with the highest assimilation value of 2.1‰. Post (2002) also strengthens the nitrogen assimilation on predators in tritrophic level-3. Nitrogen enrichment is 3.4‰ per trophic level increase in water and land ecosystems. Stable isotopes enable researchers to assess individual responses to environmental conditions, assess the potential importance of gut symbionts for insect nutrition, detect biosynthetic pathways based on labeled compounds, gain complementary dietary information from other chemical signatures (e.g., fatty acids), and investigate how (Quinby et al. 2020).

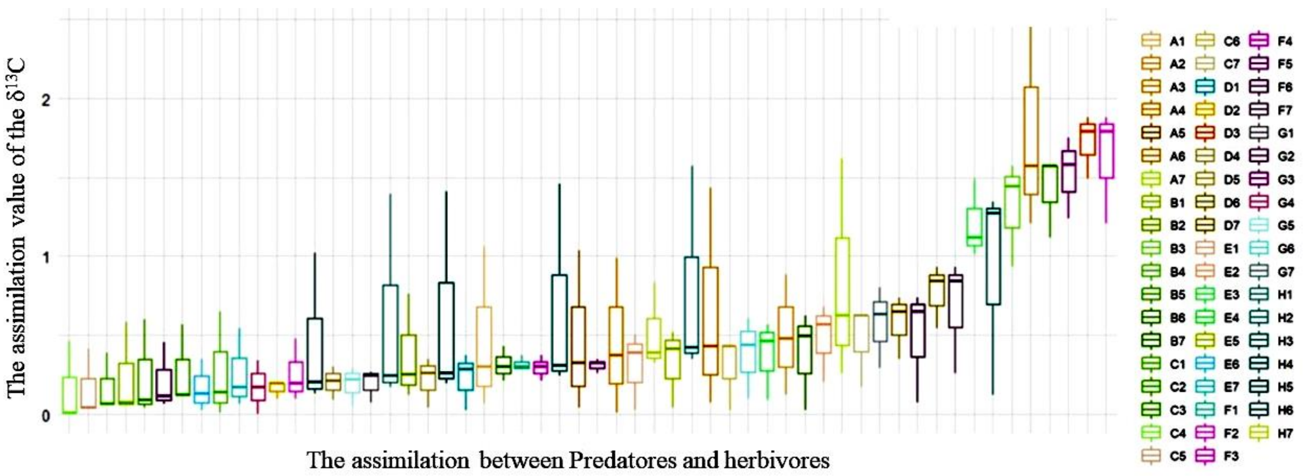

Figure 4. The assimilation value of $\delta^{13}C$ ratio between predator and herbivores in the ratoon sugarcane ecosystem. (A1: *Exypnus* and *C. sacchariphagus*, B1: *Exypnus* and *S. excerptalis,* C1: *Exypnus* and *L. stigma, D1: Exypnus* and *A. tegalensis,* E1: *Exypnus* and *S. sacchari,* F1: *Exypnus* and *Oligonychus,* G1: *Exypnus* and *C. lanigera*, H1: *Exypnus* and *Phyllophaga,* A2: *Lycosa* and *C. sacchariphagus*, B2: *Lycosa* and *S. excerptalis,* C2: *Lycosa* and *L. stigma, D2: Lycosa* and *A. tegalensis,* E2: *Lycosa* and *S. sacchari,* F2: *Lycosa* and *Oligonychus,* G2: *Lycosa* and *C. lanigera*, H2: *Lycosa* and *Phyllophaga,* A3: *Tetragnatha* and *C. sacchariphagus*, B3: *Tetragnatha* and *S. excerptalis,* C3: *Tetragnatha* and *L. stigma, D3: Tetragnatha* and *A. tegalensis,* E3: *Tetragnatha* and *S. sacchari,* F3: *Tetragnatha* and *Oligonychus,* G3: *Tetragnatha* and *C. lanigera*, H3: *Tetragnatha* and *Phyllophaga,* A4: *Dolychoderus* and *C. sacchariphagus*, B4: *Dolychoderus* and *S. excerptalis,* C4: *Dolychoderus* and *L. stigma, D4: Dolychoderus* and *A. tegalensis,* E4: *Dolychoderus* and *S. sacchari,* F4: *Dolychoderus* and *Oligonychus,* G4: *Dolychoderus* and *C. lanigera,* H4: *Dolychoderus* and *Phyllophaga,* A5: *Componothus* and *C. sacchariphagus*, B5: *Componothus* and *S. excerptalis,* C5: *Componothus* and *L. stigma, D5: Componothus* and *A. tegalensis,* E5: *Componothus* and *S. sacchari,* F5: *Componothus* and *Oligonychus,* G5: *Componothus* and *C. lanigera,* H5: *Componothus* and *Phyllophaga,* A6: *Oecophylla* and *C. sacchariphagus*, B6: *Oecophylla* and *S. excerptalis,* C6: *Oecophylla* and *L. stigma, D6: Oecophylla* and *A. tegalensis,* E6: *Oecophylla* and *S. sacchari,* F6: *Oecophylla* and *Oligonychus,* G6: *Oecophylla* and *C. lanigera*, H6: *Oecophylla* and *Phyllophaga,* A7: *Anax* and *C. sacchariphagus*, B7: *Anax* and *S. excerptalis,* C7: *Anax* and *L. stigma, D7: Anax* and *A. tegalensis,* E7: *Anax* and *S. sacchari,* F7: *Anax* and *Oligonychus,* G7: *Anax* and *C. lanigera,* H7: *Anax* and *Phyllophaga).*

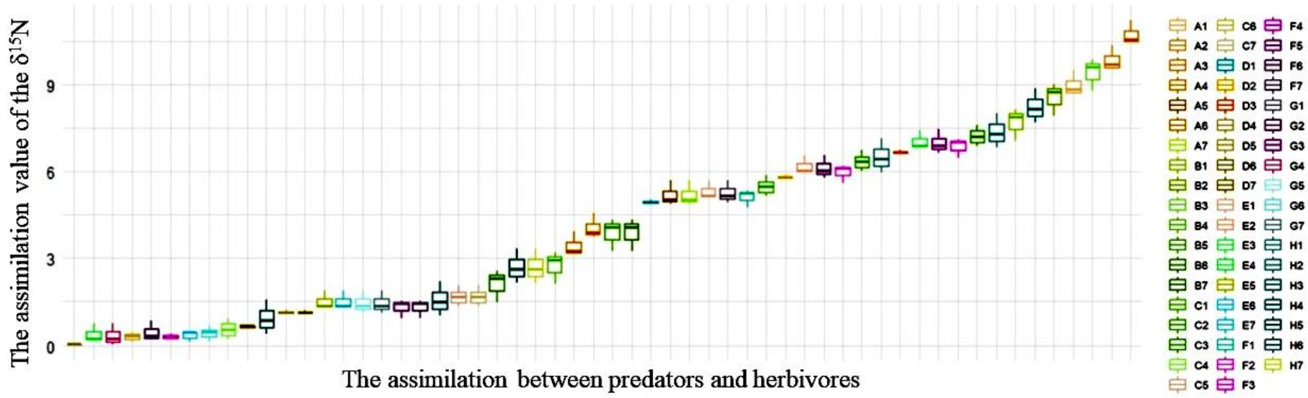

Figure 5. The assimilation value of the ratio of $\delta^{15}N$ predator to herbivores in the ratoon sugarcane ecosystem. (A1: *Exypnus* and *C. sacchariphagus*, B1: *Exypnus* and *S. excerptalis,* C1: *Exypnus* and *L. stigma, D1: Exypnus* and *A. tegalensis,* E1: *Exypnus* and *S. sacchari,* F1: *Exypnus* and *Oligonychus,* G1: *Exypnus* and *C. lanigera*, H1: *Exypnus* and *Phyllophaga,* A2: *Lycosa* and *C. sacchariphagus*, B2: *Lycosa* and *S. excerptalis,* C2: *Lycosa* and *L. stigma, D2: Lycosa* and *A. tegalensis,* E2: *Lycosa* and *S. sacchari,* F2: *Lycosa* and *Oligonychus,* G2: *Lycosa* and *C. lanigera,* H2: *Lycosa* and *Phyllophaga,* A3: *Tetragnatha* and *C. sacchariphagus*, B3: *Tetragnatha* and *S. excerptalis,* C3: *Tetragnatha* and *L. stigma, D3: Tetragnatha* and *A. tegalensis,* E3: *Tetragnatha* and *S. sacchari,* F3: *Tetragnatha* and *Oligonychus,* G3: *Tetragnatha* and *C. lanigera*, H3: *Tetragnatha* and *Phyllophaga,* A4: *Dolychoderus* and *C. sacchariphagus*, B4: *Dolychoderus* and *S. excerptalis,* C4: *Dolychoderus* and *L. stigma, D4: Dolychoderus* and *A. tegalensis,* E4: *Dolychoderus* and *S. sacchari,* F4: *Dolychoderus* and *Oligonychus,* G4: *Dolychoderus* and *C. lanigera,* H4: *Dolychoderus* and *Phyllophaga,* A5: *Componothus* and *C. sacchariphagus*, B5: *Componothus* and *S. excerptalis,* C5: *Componothus* and *L. stigma, D5: Componothus* and *A. tegalensis,* E5: *Componothus* and *S. sacchari,* F5: *Componothus* and *Oligonychus,* G5: *Componothus* and *C. lanigera*, H5: *Componothus* and *Phyllophaga,* A6: *Oecophylla* and *C. sacchariphagus*, B6: *Oecophylla* and *S. excerptalis*, C6: *Oecophylla* and *L. stigma, D6: Oecophylla* and *A. tegalensis,* E6: *Oecophylla* and *S. sacchari,* F6: *Oecophylla* and *Oligonychus,* G6: *Oecophylla* and *C. lanigera,* H6: *Oecophylla* and *Phyllophaga,* A7: *Anax* and *C. sacchariphagus*, B7: *Anax* and *S. excerptalis,* C7: *Anax* and *L. stigma, D7: Anax* and *A. tegalensis,* E7: *Anax* and *S. sacchari,* F7: *Anax* and *Oligonychus,* G7: *Anax* and *C. lanigera,* H7: *Anax* and *Phyllophaga)*

Tritrophic level three in the ratoon sugarcane agroecosystem is also filled with arthropods with the role of predator; arthropods also occupy this position with parasitoids. Parasitoids in the ratoon sugarcane agroecosystem also serve as fluctuation balancers of the herbivore population. Its existence is also expected to follow the increase in herbivore population and suppress the population addition; hence, it does not cause economic loss in the ratoon sugarcane agroecosystem. Based on the analysis of variance (ANOVA), the value of carbon ($\delta^{13}C$) ($F_{29.60}$=3.72, P=0.000001) and nitrogen ($\delta^{15}N$) ($F_{29.60}$=27.74 P=0.000001) from arthropods found with food resources potential has a significant difference. The parasitoids' carbon stable isotope value ($\delta^{13}C$) varies from 10.5 to 11.05‰. In contrast, parasitoids' nitrogen stable isotope value ($\delta^{15}N$) varies from 12.8 to 17.05‰. Carbon isotope values ($\delta^{13}C$) of parasitoids indicate commonly consumed food resources. In most of their life, parasitoids meet the carbon element from parasitism on herbivores in the agroecosystem. The carbon ($\delta^{13}C$) isotope value for parasitoids is close to zero. It proves that predators do not eat directly from sugarcane plants in their life (autotroph). In contrast, parasitoids' nitrogen stable isotope value ($\delta^{15}N$) shows their role in the ratoon sugarcane agroecosystem. Because in their life, predators mostly eat arthropods in tritrophic level-two; hence, the accumulation of nitrogen content is relatively abundant compared to herbivores or autotrophs. Nitrogen stable isotope values on arthropods are averagely above 10‰.

The role of parasitoids in the ratoon sugarcane agroecosystem is also shown by the stable isotope assimilation value of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) between parasitoids and herbivores. Based on analysis of variance (ANOVA), carbon (δ13C) assimilation value ($F_{47.96}$=2.88, P=0.000001) and nitrogen (δ15N) ($F_{47.96}$=40.51, P=0.000001) from parasitoids to herbivores has a significant difference. The value of carbon ($\delta^{13}C$) stable isotope assimilation varies with the range of 5.3 to 9.23‰ (Figure 6). While the value of nitrogen ($\delta^{15}N$) stable isotope assimilation varies with the range of 3.79 to 10.3‰ (Figure 7).

Several recent reviews have estimated that with each trophic transfer, consumers become enriched in the heavy nitrogen isotope on the order of 2.3 to 3.4. Furthermore, consumers become enriched in the heavy carbon isotope by 0.4 to 0.5 per trophic transfer. However, these estimates have been used to infer trophic interactions in a wide range of taxa (Langellotto et al. 2005). The variety of assimilation values agrees with other studies, which state that the values of carbon ($\delta^{13}C$) stable isotope assimilation on predators range between 4 to 5‰ (Ottonetti et al. 2008). The highest nitrogen assimilation value is obtained from the parasitism relationship between *Cotesia* and *C. sacchariphagus.* In the food chain in the ecosystem, enrichment of carbon and nitrogen in parasitoids happens with values of 0.5 and 3.4‰, respectively, per trophic level increase (Langellotto et al. 2005).

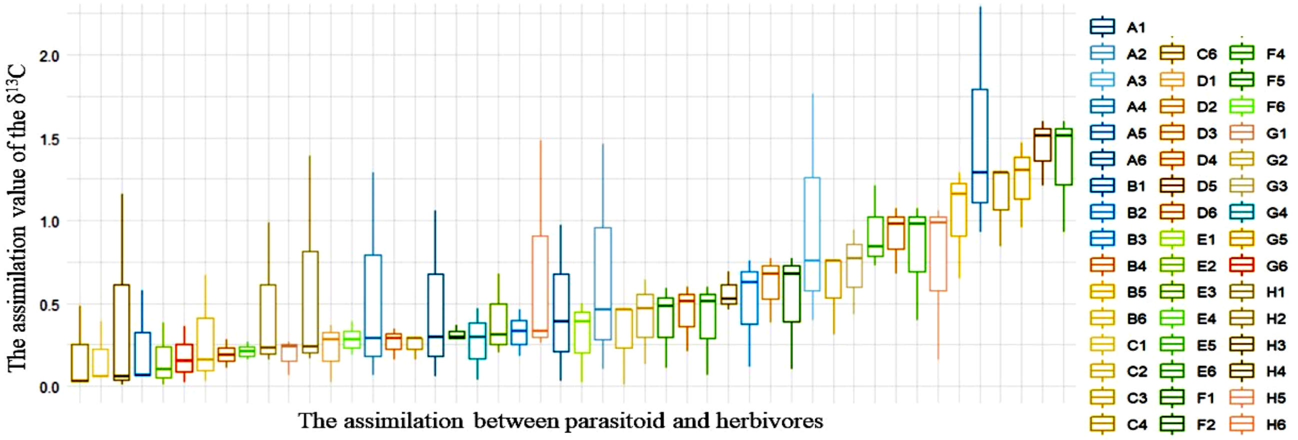

Figure 6. The assimilation value of the ratio of $\delta^{13}C$ parasitoids with herbivores in the ratoon sugarcane ecosystem. (A1: *Scolia* and *C. sacchariphagus*, B1: *Scolia* and *S. excerptalis,* C1: *Scolia* and *L. stigma, D1: Scolia* and *A. tegalensis,* E1: *Scolia* and *S. sacchari,* F1: *Scolia* and *Oligonychus,* G1: *Scolia* and *C. lanigera,* H1: *Scolia* and *Phyllophaga,* A2: *Telenomus* and *C. sacchariphagus*, B2: *Telenomus* and *S. excerptalis,* C2: *Telenomus* and *L. stigma, D2: Telenomus* and *A. tegalensis,* E2: *Telenomus* and *S. sacchari,* F2: *Telenomus* and *Oligonychus,* G2: *Telenomus* and *C. lanigera,* H2: *Telenomus* and *Phyllophaga,* A3: *Isotima* and *C. sacchariphagus*, B3: *Isotima* and *S. excerptalis,* C3: *Isotima* and *L. stigma, D3: Isotima* and *A. tegalensis,* E3: *Isotima* and *S. sacchari,* F3: *Isotima* and *Oligonychus,* G3: *Isotima* and *C. lanigera,* H3: *Isotima* and *Phyllophaga,* A4: *Chalybion* and *C. sacchariphagus*, B4: *Chalybion* and *S. excerptalis,* C4: *Chalybion* and *L. stigma, D4: Chalybion* and *A. tegalensis,* E4: *Chalybion* and *S. sacchari,* F4: *Chalybion* and *Oligonychus,* G4: *Chalybion* and *C. lanigera,* H4: *Chalybion* and *Phyllophaga,* A5: *Trichogramma* and *C. sacchariphagus*, B5: *Trichogramma* and *S. excerptalis,* C5: *Trichogramma* and *L. stigma, D5: Trichogramma* and *A. tegalensis,* E5: *Trichogramma* and *S. sacchari,* F5: *Trichogramma* and *Oligonychus,* G5: *Trichogramma* and *C. lanigera,* H5: *Trichogramma* and *Phyllophaga,* A6: *Cotesia* and *C. sacchariphagus*, B6: *Cotesia* and *S. excerptalis,* C6: *Cotesia* and *L. stigma, D6: Cotesia* and *A. tegalensis,* E6: *Cotesia* and *S. sacchari,* F6: *Cotesia* and *Oligonychus,* G6: *Cotesia* and *C. lanigera*, H6: *Cotesia* and *Phyllophaga)*

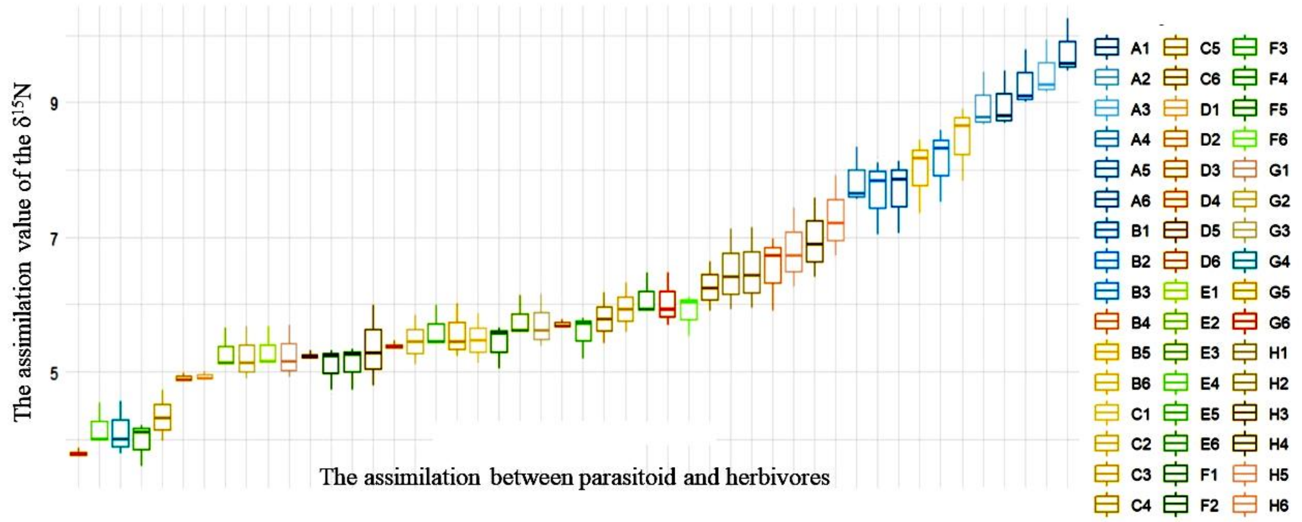

Figure 7. The assimilation value of the ratio of $\delta^{15}N$ parasitoids with herbivores in the ratoon sugarcane ecosystem. (A1: *Scolia* and *C. sacchariphagus*, B1: *Scolia* and *S. excerptalis,* C1: *Scolia* and *L. stigma, D1: Scolia* and *A. tegalensis,* E1: *Scolia* and *S. sacchari,* F1: *Scolia* and *Oligonychus,* G1: *Scolia* and *C. lanigera,* H1: *Scolia* and *Phyllophaga,* A2: *Telenomus* and *C. sacchariphagus*, B2: *Telenomus* and *S. excerptalis,* C2: *Telenomus* and *L. stigma, D2: Telenomus* and *A. tegalensis,* E2: *Telenomus* and *S. sacchari,* F2: *Telenomus* and *Oligonychus,* G2: *Telenomus* and *C. lanigera,* H2: *Telenomus* and *Phyllophaga,* A3: *Isotima* and *C. sacchariphagus*, B3: *Isotima* and *S. excerptalis,* C3: *Isotima* and *L. stigma, D3: Isotima* and *A. tegalensis,* E3: *Isotima* and *S. sacchari,* F3: *Isotima* and *Oligonychus,* G3: *Isotima* and *C. lanigera,* H3: *Isotima* and *Phyllophaga,* A4: *Chalybion* and *C. sacchariphagus*, B4: *Chalybion* and *S. excerptalis,* C4: *Chalybion* and *L. stigma, D4: Chalybion* and *A. tegalensis,* E4: *Chalybion* and *S. sacchari,* F4: *Chalybion* and *Oligonychus,* G4: *Chalybion* and *C. lanigera,* H4: *Chalybion* and *Phyllophaga,* A5: *Trichogramma* and *C. sacchariphagus*, B5: *Trichogramma* and *S. excerptalis,* C5: *Trichogramma* and *L. stigma, D5: Trichogramma* and *A. tegalensis,* E5: *Trichogramma* and *S. sacchari,* F5: *Trichogramma* and *Oligonychus,* G5: *Trichogramma* and *C. lanigera,* H5: *Trichogramma* and *Phyllophaga,* A6: *Cotesia* and *C. sacchariphagus*, B6: *Cotesia* and *S. excerptalis,* C6: *Cotesia* and *L. stigma, D6: Cotesia* and *A. tegalensis,* E6: *Cotesia* and *S. sacchari,* F6: *Cotesia* and *Oligonychus,* G6: *Cotesia* and *C. lanigera*, H6: *Cotesia* and *Phyllophaga).*

The roles of arthropods in the ratoon sugarcane agroecosystem can be figured out by looking at the stable isotopes $\delta^{13}C$ and $\delta^{15}N$. The ratio of arthropod carbon assimilation to sugarcane ranges from-1.4 to-5.45. While the ratio of arthropod nitrogen assimilation to sugarcane ranges from 3.86 to 39.7. On both predators and parasitoids, the values of the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ are different. For predators, the stable carbon isotope value ($\delta^{13}C$) ranges from-10.14 to-11.62. The stable nitrogen isotope value ($\delta^{15}N$) for predators ranges from 9.17 to 18.1%. Parasitoids have stable carbon isotope values ($\delta^{13}C$) that range from 10.5 to 11.05. Parasitoids have stable nitrogen isotope values ($\delta^{15}N$) that range from 12.8 to 17.05. The value of carbon ($\delta^{13}C$) stable isotope assimilation varies from 0.006 to 1.38 between herbivores and predators. While the value of stable isotope assimilation of nitrogen ($\delta^{15}N$) varies from 0.33 to 10.3. Also, the value of carbon ($\delta^{13}C$) stable isotope assimilation varies from 5.3 to 9.23 between herbivores and parasitoids. Isotope content ($\delta^{13}C$) shows where arthropods get their food in an agroecosystem, while isotope value ($\delta^{15}N$) shows what roles arthropods play in a ratoon sugarcane agroecosystem. Both predators and parasitoids have carbon-stable isotope values that are close to zero. Even though the stable nitrogen isotope ($\delta^{15}N$) values of arthropods are usually above 10%, it is thought that they are either predators or parasitoids. The relationship between producers (sugarcane) and consumers (detritivores, herbivores, and predators) affects the ecosystem's stability and the amount of sugar cane that can be grown. Recognizing how energy moves between predators and parasitoids through stable isotopes can be used to protect and improve natural enemies to improve ecosystem services and slow the growth of herbivores.

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