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# Stable isotopes in monitoring terrestrial arthropods

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Monitoring of arthropods focuses typically on changes in population and range size over time. Yet, there are a myriad of other aspects that could and should be monitored under the ongoing global and local environmental change. Stable isotope analysis, widely employed in short-term ecological studies, has potential in long-term monitoring of arthropods. Here we discuss the use of stable isotopes in monitoring terrestrial arthropods, provide some empirical examples of the use of bulk tissue samples in stable isotope analysis, and outline future directions in using compound-specific stable isotope analysis in monitoring. We performed a literature search for 2012–2021 to see if stable isotopes have been specifically used in monitoring of terrestrial arthropods. The literature shows that stable isotopes have been successfully used to reveal ecological phenomena (dispersal, trophic interactions, resource use) that would have been difficult or impossible to detect by other means. Yet, stable isotopes have been underused in arthropod monitoring programs, but the growing number of basic studies on stable isotope ecology and methodology provides crucial basis needed for developing monitoring programs. Stable isotopes provide technically, economically and ecologically feasible addition to the traditional monitoring methods of terrestrial arthropods.

## KEYWORDS

**bulk tissue samples, compound-specific stable isotopes, dispersal, insect monitoring, resource use, trophic interactions**

## Introduction

The ongoing global and local environmental change calls for a variety of objectives and methods to monitor arthropods. Monitoring can be defined as continuous recording of some response variable over time, as opposed to short-term ecological studies, which often focus on explaining phenomena; but the difference is of degree, not kind. Traditionally, monitoring of arthropods has focused on changes in population and range size over time. Yet, there are a myriad of other aspects (e.g., harmful substances, body condition, resource use, origin of migrants) that could and should be monitored to fully capture the effect of environmental change on populations. Stable isotope analysis (SIA) is one promising avenue for monitoring arthropod populations at many temporal and spatial scales. SIA is particularly useful in revealing changes in resource use, energy

and nutrient flow, and dispersal (movement between populations) and migration (regular back-and-forth movement between habitats or regions) (Hood-Nowotny and Knols, 2007; Hobson and Wassenaar, 2019; Quinby et al., 2020), which all are subjected to change under the global and local environmental changes. Although SIA has been extensively used in ecological studies (Fry, 2006), especially in aquatic ecosystems, its use with terrestrial arthropods has lagged behind (Boecklen et al., 2011; Quinby et al., 2020).

Despite the increasing number of studies using SIA, their use in monitoring has been rarely discussed. The traditional bulk tissue sample isotope analysis is widely used and suitable for many research purposes, but the recent development in compound-specific stable isotope analysis (CSIA) provides novel approaches for monitoring (Riekenberg et al., 2021). In this perspective paper, we discuss the use of bulk tissue SIA in monitoring arthropod populations and outline some future directions in monitoring using CSIA.

## Existing literature

We did a literature search to identify recent studies that have used stable isotopes (SI) in monitoring of terrestrial arthropods (see Quinby et al., 2020 for a comprehensive review on SI ecology in arthropods). We searched for journal articles in the Web of Science on the 6<sup>th</sup> of April 2022, using keywords “Stable isotopes\* AND ”insect\*” IN Abstract during 2012–2021, which gave 366 articles. Replacing “insect\*” with “arthropod\*” gave 103 articles with some overlap. We excluded articles on aquatic organisms as well as articles with paleontological, physiological or biochemical focus. Many articles focused on aquatic-terrestrial interface, i.e., included terrestrial taxa, and these were included. This left us with 136 articles (Supplementary material), which should give an adequate, although not a complete picture of the research.

We found very few papers in which monitoring arthropod populations was the main focus; yet, they provide important basic information that are needed in developing monitoring programs. Basically, all articles (97%) focused on two aspects of arthropod ecology: dispersal and trophic interaction (energy flow and resource use) or both. In the following chapters, we outline main methodological approaches in SI ecology and give examples how they could supplement monitoring programs.

## Natural abundance vs. enrichment approach

Two different kinds of approaches exist in SI ecology. Natural abundance studies use naturally occurring differences in isotope values and enrichment studies use human-induced labeled compounds enriched in particular isotopes of demand,

which are added to the study system and followed. Both of these approaches could be used in monitoring.

*Natural abundance* approach in SI studies is based on the differences in organisms' SI ratios, i.e., SI “signatures” can be considered natural markings. The approach resembles studies of genetic variation but is used to answer questions that would be impossible to answer using molecular methods. Depending on the element, SI ratio differences reflect variation in energy sources (dietary and basal sources), trophic position or different geographical origins. Because the distribution of isotopes in nature, at least in magnitude, is rather well known, or can be analyzed, this is a rather straightforward method.

*Enrichment approach* is closely related to traditional mark-release-recapture (MRR) techniques. There are many commercially available SI-enriched compounds that have higher concentrations of the rarer isotope than the natural background. One common way used in studies of agricultural pests is to label the host plant. Various plant enrichment techniques have been developed, using soil watering and plant culture in isotopically enriched soil (Unsicker et al., 2005), gas-tight labeling chambers (Hood et al., 2004), foliage spraying (Hood and Blair, 2001), foliage brushing (Putz et al., 2011), and stem injection (Russell and Fillery, 1996; Mahieu et al., 2009). These techniques vary in the dynamics of marker absorption, the ability to label a particular plant species or plant part, the loss of labeling solution, and the required amount of marker. Thus, unlike in natural abundance approach, enrichment approach requires more basic research to develop appropriate enrichment techniques for study design. Furthermore, basic understanding is needed about the marker's adequate transfer to different body parts and its persistence in the focal arthropod (i.e. turnover rate).

For example, Porras et al. (2020) studied the <sup>15</sup>N enrichment of the spotted lanternfly (Hemiptera) and demonstrated the usability of SI approach in monitoring. They concluded that the advantages of labeling the spotted lanternfly through host plant-enrichment are: (1) the method is metabolically benign allowing insects to have the necessary nutritional intake to grow and complete their life cycle; (2) the spray application system is easy to perform; and (3) the isotope creates a robust label in both plants and the spotted lanternfly, making <sup>15</sup>N an ideal tool for field studies; (4) there was no effect on insect behavior; and (5) the method is cheap as it requires minimal infrastructure for sample preparation, and sample analysis is inexpensive compared to molecular methods.

## Bulk tissue sample stable isotope analyses

Here we discuss two themes that we found from the literature and which are relevant to monitor: trophic interactions (energy flow and resource use) and movement (dispersal and migration).

## Trophic interactions

Changes in resource use is crucial in understanding and predicting pest dynamics. In many arthropods, resource use is difficult to study by direct observation. Isotopic techniques have distinct advantages over traditional techniques such as gut content analysis and observation, as these allow for long-term monitoring and are generally less time consuming.

Torniainen and Komonen (2021) studied the trophic position of social wasps (Vespinae), often assumed generalist predators. They found significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among species, which were consistent among locations. This suggests fine-scale differentiation in food sources within the predatory trophic level, which contrasts with the assumed generalist feeding behavior of wasps. The same isotope analyses were used to monitor changes in food sources of two *Vespula vulgaris* colonies over season. Both colonies showed simultaneous changes in isotope values in late season, suggesting change in food sources. The spatially consistent isotope values of species provides easier detection of abnormalities during monitoring, because the initial reference values are known. Furthermore, the fine-scale temporal changes in isotope value over season suggest that isotopes could also be used to monitor long-term changes in resource use, resulting from human-induced environmental change.

Morente and Ruano (2022) studied trophic interactions of arthropod community in olive grove via  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analysis. Results proved novel understanding of the arthropod community resource use, plasticity and natural pest control against olive fruit fly *Bactrocera oleae* and olive moth *Prays oleae* at the expense of olive psyllid *Euphyllura olivina* abundance. Similarly, via  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analysis, Penick et al. (2015) revealed that ants in urban habitat have strong resource plasticity by changing from natural food source to human food inputs. These two latter examples show what kind of interactions occur in an olive grove arthropod community, and the impact of human provided exogenous food resource. These events could easily remain unknown and aside monitoring without the use of SIA. With arthropods the drawback of all SI methods is that sampling generally requires killing, although in some cases dead specimens or pupal remains can be used.

## Movement

Bulk tissue SIA can be combined with mark-release-recapture techniques to monitor dispersal and migration. Traditional insect marking techniques, such as fluorescent dyes, protein labels, radioactive labels and tags have various limitations; notably low marker retention and inability to mark individuals at source.

*Natural abundance studies* are used when the origin of migrants is unknown and other marking techniques are not

applicable, or it is impossible to mark them (Brattström et al., 2018; Qin and Shi, 2020). The origin of migrants may also vary in time, which as such may be worth monitoring (Hobson et al., 1999). For example, understanding transgenerational and highly adaptive migratory behavior is crucial when interpreting life cycle dynamics of insect pests. Torniainen and Mikonranta (2018) studied the origin of northern European silver Y moths, *Autographa gamma*, in Finland. The difference between spring and autumn generation  $\delta^2\text{H}$  values indicated different geographical natal origins. The probability surface map, created by comparing moth wing  $\delta^2\text{H}$  against global precipitation water  $\delta^2\text{H}$  with appropriate transfer function, shows that the spring generation probably emerged in central Europe. One unutilized benefit of SIA is the use of museum samples, which can reveal long-term changes in dispersal and migration (van Hardenbroek et al., 2012). For example, Torniainen and Mikonranta (2018) found a negative correlation between the silver Y moth wing  $\delta^2\text{H}$  values and the migrants' capture year suggesting that a warming climate may have driven the transgenerational migratory stages northwards during the last century. The natural abundance isotope technique could be used to monitor the origin of invasive species and to determine whether insects are transitory migrants or belong to established resident populations.

*Enrichment studies* are used when the environment does not provide isotopic differences in the study system. This method has similarities to traditional MRR studies. Pollier et al. (2016) developed a method of nectar labeling based on the injection of labeled sugar solution into the plant stem. This allowed them to analyze the nectar uptake by parasitoids. After injection they analyzed the transfer of  $^{13}\text{C}$  from the sugar solution into extrafloral nectaries, the uptake of labeled nectar by parasitoids under laboratory conditions, and in the field the ability of the method to discriminate between labeled parasitoids and unlabeled parasitoids to track parasitoid movements. The extrafloral nectar of all test plants was  $^{13}\text{C}$ -labeled. Most (66%) of the parasitoids were identified as marked after 96 h of exposure to labeled plants in the laboratory. Pollier et al. (2016) also detected labeled parasitoids in the field trial, but the detection rate was only 1%. The experiments demonstrate that the method is appropriate to label extrafloral nectar and parasitoids feeding on this labeled nectar. Further research is needed on the amount of labeled extrafloral nectar required to obtain a sufficient marker level to monitor parasitoid movements in the field.

## Compound-specific stable isotopes

The use of bulk tissue SIs in natural abundance studies is frequently hindered by extensive variability and overlapping values of primary producer baseline values or among potential food items. Thus, monitoring of arthropods with SIs is

practically impossible or at best very unreliable. To overcome this problem, compound-specific stable isotope analysis (CSIA) of macromolecules (e.g., fatty acids and amino acids) are becoming an indispensable tool for elucidating ecological questions, and increasing the applicability of SI techniques in monitoring. Unlike in bulk isotope analysis, specific macromolecules may differ in synthesis pathway or their routing from specific dietary sources providing possibly unique isotope ratio. Variety of CSIA applications exist, but macromolecules mostly used are amino acids (AAs) and fatty acids (FAs).

## Amino acid CSIA

Amino acid CSIA studies in terrestrial arthropods has focused on measuring trophic position of organisms using approach based on nitrogen isotope fractionation in two AAs, glutamic acid and phenylalanine. Glutamic acid can be considered as “trophic AA” and shows predictable increase of  $\delta^{15}\text{N}$  values during reactions that cleaves the carbon–nitrogen bond, whereas phenylalanine, considered as “source AA” shows little change in  $\delta^{15}\text{N}$  values during conversion to tyrosine that neither forms nor cleaves the carbon–nitrogen bond (Chikaraishi et al., 2011). This method is rather well established and has the greatest potential of CSIA methods to become tool for monitoring arthropods in the near future. It should be noted that there are several other potentially useful source and trophic AAs, but so far, their use in arthropod studies has been limited. Consequently, these two type AAs enable trophic position estimation based on the nitrogen isotope values only from target organism and therefore has an advantage over bulk stable isotope analysis, where basal  $\delta^{15}\text{N}$  values of the study system need to be known. This could be beneficial for monitoring arthropods since their basal resources are often unknown, they are difficult to sample or they signatures are highly variable in time and space. The method is also considered to provide more accurate trophic position estimates compared to bulk stable isotopes, which increases its applicability in monitoring of small-scale changes in resource use. For example, Steffan et al. (2019) investigated trophic position of all major bee families and showed that they are not strict herbivores, but their trophic position within each family was significantly greater than 2, varying between 2.1 and 3.1. Increased trophic position seemed to be a consequence of pollen born microbes of which many have shown to be symbiotic by conditioning and increasing the nutritional quality of the pollen for the bees. AA CSIA could provide relatively simple monitoring tool to study such symbiotic relationships and similar processes in space and time, which are sensitive to anthropogenic disturbances such as fungicides.

Main drawback of the method is that it cannot be applied to very complex food webs where the basal resources of the

study species are derived through multiple pathways (e.g., terrestrial C3 and C4 plants, and aquatic photoautotrophs), because isotopic difference between “trophic AAs” and “source AAs” varies among basal resources. This should not be a major problem in monitoring, which often focuses on single species, which are also monophagous (e.g., agricultural pests or threatened species). Nevertheless, in complex food webs dual or trial CSIA of AAs ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  or  $\delta^2\text{H}$ ) could overcome this issue by quantifying mixing ratio among different sources using  $\delta^{13}\text{C}$  and/or  $\delta^2\text{H}$  of amino acids. Indeed, carbon CSIA of essential AAs has been tested in conjugation with nitrogen CSIA AAs to study trophic relationships in complex soil food webs (Pollierer et al., 2019), although to our knowledge true integration of two elements to quantify mixing ratio among different sources has not been accomplished yet. Nevertheless, carbon CSIA of essential AA seem to differentiate main basal energy sources (fungi, bacteria and plants) of soil food webs relatively well (Larsen et al., 2009; Pollierer et al., 2019) and possibly enables monitoring of arthropod food web interactions in different type of systems (Pollierer et al., 2019). While use of AA CSIA in arthropods monitoring has the greatest potential in estimation of trophic position of individuals, there are new emerging applications such as using AA  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^2\text{H}$  in monitoring animal movements in high resolution (McMahon and Newsome, 2019).

## Fatty acid CSIA

Fatty acid CSIA can be done from total fatty acids or from specific group of fractionated lipids such as polar phospholipids and neutral lipids (Twining et al., 2020). Polar and neutral lipids have different functions in the living tissue (cell membrane vs. storage lipids) and can provide answers to variety of question depending on which group of lipids (or total lipids) have been analyzed for stable isotopes. Similar to CSIA AAs, the benefit of using CSIA FAs over bulk tissue analysis in monitoring is its ability to detect isotopic differences between basal sources. CSIA FA can provide insights to lipid metabolism and incorporation, and in particular provide means to monitor dietary origin with essential FAs (Twining et al., 2020). Most CSIA FA studies have focused on analyses of the carbon isotope ratios, but methodological advances in recent years have made it possible to analyze also hydrogen isotopes.

For example, Mathieu-Resuge et al. (2021) investigated the trophic transfer of fatty acids from emergent aquatic and terrestrial insects to spiders at varying distances from the shoreline of a subalpine lake, using CSIA FA carbon and hydrogen. They find out that diet sources clearly differed in  $\delta^2\text{H}$  values of selected fatty acids depending on the spider's habitat. Difference was less evident from the  $\delta^{13}\text{C}$  values of the fatty

acids. They concluded that the dual use of  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  of FA is a promising tool for food web studies to characterize various diet sources and pathways to consumers. Indeed, such a tool might provide new avenues to monitor dietary changes in terrestrial food webs, which has been notoriously difficult with traditional isotope methods, due to overlapping isotope signatures. However, there is still a need for well-designed laboratory experiment to better understand fractionation of  $\delta^2\text{H}$  in FAs before these tools are applicable for monitoring resource use of terrestrial arthropods.

## Conclusion

Stable isotopes have been widely used in short-term ecological studies and they are a promising avenue for long-term monitoring of arthropods. Here we have identified six points, which are relevant for arthropod monitoring: (1) The use of SIs requires methodological development and basic information about inherent patterns in nature, which increases their applicability from the technical, economic and ecological point of view. (2) SIA has proven useful in monitoring resource use of terrestrial taxa that would be otherwise difficult to monitor, such as soil fauna or highly mobile generalist foragers. (3) SIA is a useful addition to the methodological pallet for studying arthropod dispersal and migration. It can reveal historical and large-scale phenomena that would be otherwise difficult or impossible to monitor. (4) SIA has also proven useful to monitor ecosystem linkages, such as those between aquatic and terrestrial systems. (5) SIA may be particularly useful in detecting changes in energy flows (e.g., resource use and dispersal) in areas where anthropogenic activities alter bio-geochemical cycles, introduce chemical compounds and hence alter the resource base for arthropods. (6) CSIA is increasing the applicability of SIA in monitoring because it applies specific macromolecules, which may differ in synthesis pathway compared to bulk tissue providing potential for more detailed and unique isotope ratio; thus, it does not always require sampling reference material, which make monitoring more cost-effective.

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## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

## Author contributions

AK got the initial idea for the manuscript. All authors contributed to the writing of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.969595/full#supplementary-material>

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