

Methods in Ecology and Evolution



Using stable isotope analysis to answer fundamental questions in invasion ecology: progress and prospects

Journal:	<i>Methods in Ecology and Evolution</i>
Manuscript ID	MEE-19-04-294.R1
Manuscript Type:	Review
Date Submitted by the Author:	n/a
Complete List of Authors:	<p>McCue, Marshall; Sable Systems International</p> <p>Javal, Marion; Stellenbosch University, Conservation Ecology and Entomology</p> <p>Clusella Trullas, Susana; Stellenbosch University Faculty of Science, ;</p> <p>Le Roux, Jaco; Stellenbosch University, Department of Botany and Zoology</p> <p>Jackson, Michelle; Imperial College London,</p> <p>Ellis, Allan; Stellenbosch University, Botany & Zoology Dept.</p> <p>Richardson, David (Dave); Stellenbosch University, Centre for Invasion Biology (CIB)</p> <p>Valentine, Alex; Stellenbosch University Faculty of Science, Dept of Botany and Zoology</p> <p>Terblanche, John; University of Stellenbosch, Conservation Ecology & Entomology</p>
Keywords:	<p>Laboratory methods < Molecular Biology, Food webs < Community Ecology, Species interactions < Community Ecology, Invasives (Population Ecology) < Population Ecology, Comparative analysis < Evolutionary Biology, Restoration < Applied Ecology, Invasives (Applied Ecology) < Applied Ecology, Agricultural systems < Applied Ecology</p>
Abstract:	<p>1. What makes some species successful invaders while others fail, and why some invaders have major impacts in invaded ecosystems are pivotal questions that are attracting major research effort. The increasing availability of high resolution, georeferenced stable isotope landscapes ('isoscapes'), coupled with the commercialization of stable isotope-enriched tracer molecules and the development of new analytical approaches, is facilitating novel applications of stable isotope techniques in ecology. We can now address ecological questions that were previously intractable.</p> <p>2. We review and discuss how stable isotope analysis (SIA) can complement fundamental research themes in the study of biological invasions, especially in answering questions relating to the physiological and ecological mechanisms underlying invasion processes and invader impacts.</p> <p>3. SIA was first used for simply describing the diet of invaders but, more recently, SIA-informed metrics of population and community trophic structure have been advanced. These approaches now permit the comparison of diets across space and time and provide quantitative tools</p>

	<p>to compare food webs across different stages of invasion.</p> <p>4. SIA has also been pivotal in quantifying competition for resources between native and non-native species (e.g., competition for food, water, or nutrient use). Specific questions related to modes of dispersal (e.g., origin and distance/direction traveled) and mechanisms of establishment can also be addressed using SIA in diverse taxa.</p> <p>5. An overarching goal is to highlight examples of recent studies that have used SIA in key areas of invasion ecology and use these to synthesize testable predictions where SIA could be applied to future studies. We conclude by highlighting several paths forward and describing how unresolved challenges in quantifying the rates, impacts, and mechanisms underlying invasions could potentially benefit from the use of SIA.</p>

REVIEW

Using stable isotope analysis to answer fundamental questions in invasion ecology: progress and prospects

Marshall D. McCue^{1,2}, Marion Javal², Susana Clusella-Trullas³, Johannes J. Le Roux^{3,4}, Michelle C. Jackson^{3,5}, Allan G. Ellis⁶, David M. Richardson³, Alex J. Valentine⁶ & John S. Terblanche²

¹Sable Systems International, Las Vegas, Nevada, USA

²Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Stellenbosch University, South Africa

³Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, South Africa

⁴Department of Biological Sciences, Macquarie University, New South Wales, Australia

⁵Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot, UK

⁶Department of Botany and Zoology, Stellenbosch University, South Africa

Correspondence

John Terblanche Email: jst@sun.ac.za

ABSTRACT

1. What makes some species successful invaders while others fail, and why some invaders have major impacts in invaded ecosystems are pivotal questions that are attracting major research effort. The increasing availability of high resolution, georeferenced stable isotope landscapes ('isoscaples'), coupled with the commercialization of stable isotope-enriched tracer molecules and the development of new analytical approaches, is facilitating novel applications of stable isotope techniques in ecology. We can now address ecological questions that were previously intractable.
2. We review and discuss how stable isotope analysis (SIA) can complement fundamental research themes in the study of biological invasions, especially in answering questions relating to the physiological and ecological mechanisms underlying invasion processes and invader impacts.
3. SIA was first used for simply describing the diet of invaders but, more recently, SIA-informed metrics of population and community trophic structure have been advanced. These approaches now permit the comparison of diets across space and time and provide quantitative tools to compare food webs across different stages of invasion.
4. SIA has also been pivotal in quantifying competition for resources between native and non-native species (*e.g.*, competition for food, water, or nutrient use). Specific questions related to modes of dispersal (*e.g.*, origin and distance/direction traveled) and mechanisms of establishment can also be addressed using SIA in diverse taxa.
5. An overarching goal is to highlight examples of recent studies that have used SIA in key areas of invasion ecology and use these to synthesize testable predictions where SIA could be applied to future studies. We conclude by highlighting several paths forward and describing how unresolved challenges in quantifying the rates, impacts, and mechanisms underlying invasions could potentially benefit from the use of SIA.

46 **KEYWORDS**

47 Adaptation, biological invasions, breath testing, competition, dispersal ecology, ecological
48 physiology, food webs, invasion dynamics, niche overlap

49

For Review Only

51 **1. INTRODUCTION**

52 **1.1 Biological invasions**

53 The phenomenon of biological invasion involves the transport of organisms through
54 human activity to areas outside their current native range (as defined by adaptation, natural
55 dispersal mechanisms and biogeographical barriers) and their performance in the novel range,
56 including their ability to survive, establish, reproduce, spread, proliferate, and interact with
57 resident biota (Hui & Richardson 2017).

58 Invasions of plant and animal species are taking place at unprecedented rates in every
59 type of ecosystem, with no apparent saturation in the number of species being introduced
60 (Westphal *et al.* 2007; Roques 2010; Roques *et al.* 2016; Seebens *et al.* 2017). Substantial
61 progress has been made in elucidating many facets of invasion ecology (Richardson 2011; Hui
62 & Richardson 2017). Despite this, the rapid changes in community structure and ecosystem
63 function that often follow an invasion are highly variable, context-dependent and are, in most
64 cases, poorly understood (Parker *et al.* 1999; Strayer *et al.* 2006; Kenis *et al.* 2009; Jeschke *et*
65 *al.* 2014; Kumschick *et al.* 2015; Bellard, Cassey & Blackburn 2016). Huge challenges remain
66 with respect to understanding the fundamental mechanisms by which invasions occur and
67 characterizing the specific ecological impacts invaders have on ecosystems.

68 The burgeoning literature on invasion ecology has generated many ‘invasion
69 frameworks’ that classify and categorize the key mechanisms and processes that mediate
70 invasions [*e.g.*, (Jeschke & Heger 2018)]. Biological invasions are usually not single discrete
71 events, but rather involve a progression of sequential events. At each stage of an invasion,
72 introduced populations must overcome a series of geographic, physiological, and ecological
73 barriers (Simberloff & Von Holle 1999; Richardson *et al.* 2000; Allendorf & Lundquist 2003;
74 Blackburn *et al.* 2011). The effectiveness of these barriers may be significantly reduced if (1) a
75 species is pre-adapted or otherwise well suited to climatic and edaphoclimatic conditions in the
76 novel range (Curnutt 2000); (2) a species experiences advantages stemming from enemy

release (Maron & Vila 2001; Keane & Crawley 2002) or cointroduction of beneficial partners (Le Roux *et al.* 2017); or (3) human-induced changes facilitate invasion (Hufbauer *et al.* 2012). Regardless of the precise mechanisms, it is increasingly evident that understanding these complex processes requires researchers to employ a wide range of approaches (Hui & Richardson 2017; Vas *et al.* 2017).

1.2 Stable isotope analysis

Stable isotope analysis (SIA) provides a powerful tool to explore many of the mechanisms implicated in invasion dynamics, and for testing many hypotheses and theories in invasion ecology. Stable isotopes are not radioactive and naturally occur in every environment and organism. The isotopic signature of a sample refers to the proportion of different atoms of the same element that have different atomic masses found in tissue or a substance. These signatures can be influenced by photosynthetic pathway, diet composition, water sources, and various environmental conditions. Stable isotopes common to all organisms [carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$), sulfur ($^{34}\text{S}/^{32}\text{S}$), hydrogen ($^2\text{H}/^1\text{H}$), and oxygen ($^{18}\text{O}/^{16}\text{O}$)] can therefore be used to measure dispersal patterns and pathways of invasion, quantify resource investment into growth and reproduction, and characterize the effects of invasions throughout food webs.

SIA has become an increasingly popular tool among ecologists, and isotopic ecology has become a productive research field in its own right (Hobson & Wassenaar 1999; Martinez del Rio *et al.* 2009). Variation in naturally occurring stable isotopes (Box 1) can be used to study food web dynamics in diverse ecosystems and has long been appreciated in general ecology (Gannes, O'Brien & Martinez del Rio 1997; Martinez del Rio *et al.* 2009). Although several studies have employed SIA to investigate biological invasions [reviewed in (Bodey, Bearhop & McDonald 2011)] its use remains sporadic [but see (Kamenova *et al.* 2017)]. The increasing availability of georeferenced, high-resolution stable isotope landscapes ['isoscapes'; *sensu* (West, Sobek & Ehleringer 2008; West *et al.* 2010; Bowen *et al.* 2014; Cheesman & Cernusak

2016)] [e.g., groundwater (West, February & Bowen 2014); marine (Magozzi *et al.* 2017); and precipitation (Terzer *et al.* 2013)] and the commercialization of stable isotope-enriched organic 'tracer' molecules (e.g., monosaccharides, amino acids, fatty acids) for artificial enrichment studies (e.g., Cambridge Isotope Laboratories, ISOTEC-Sigma Aldrich) provide new applications for SIA.

It is currently possible to artificially enrich thousands of different molecules with stable isotopes (McCue & Welch Jr 2016) which, when purified, can be used as tracer molecules to isotopically enrich plants and animals to track the flow of specific resources or propagules through ecosystems. The past two decades have seen the cost of commonly used tracers decrease dramatically; highly enriched ^{13}C -glucose, for example, is less \$100 per gram (Hood-Nowotny & Knols 2007). Over the same time the development of laser-based isotope analyzers allows users to make measurements in near-real time with higher throughput and lower costs than ever (Voigt 2009; McCue & Welch Jr 2016; Welch Jr *et al.* 2016). The direct integration of SIA with chromatography system and other molecular separation techniques now enables researchers to conduct compound-specific SIA (quantification of stable isotope distributions among individual amino acids or fatty acids in a tissue sample) [e.g., (Cifuentes & Salata 2001; McClelland & Montoya 2002; Chamberlain *et al.* 2004; Chikaraishi, Naraoka & Poulson 2004; McCarthy *et al.* 2007)].

To our knowledge no studies have documented reduced fitness or deleterious long-term effects of stable isotope tracers in free-living organisms [unlike radioactive isotopes e.g., (Braden, Lembcke & Caspary 2007)]. The analytical methods for measuring and reporting stable isotope values are well established but are beyond the scope of this review; readers interested in learning such fundamentals can find several excellent reviews on the topic (Peterson & Fry 1987; Farquhar, Ehleringer & Hubick 1989; Gannes, Martinez del Rio & Koch 1998; Hayes 2001; Coplen *et al.* 2002; McKechnie 2004; Thompson *et al.* 2005; McCue 2011; O'Brien 2015).

This paper summarizes some of the most elegant and informative uses of SIA in field and laboratory studies and describes new applications for SIA to answer fundamental questions in invasion science with focus on examples of how such approaches could elucidate the physiological and ecological mechanisms underlying invasions and invader impacts. We also highlight how researchers can use differences in the natural abundances of stable isotopes as well as purified tracer molecules to address key questions in invasion ecology.

2. DISPERSAL

2.1 Natural abundance approaches

Dispersal is a critical aspect of invasions both for overcoming barriers to becoming established in new environments and for determining the subsequent geographical spread of an invasive species once it has become established (Trakhtenbrot *et al.* 2005). Stable isotopes (particularly ^2H and ^{18}O) have been extensively used to trace origins and migration patterns of individuals across large spatial scales (Hobson 1999; Graves, Romanek & Rodriguez-Navarro 2002; Rubenstein & Hobson 2004; Inger & Bearhop 2008; Heinrich & Collins 2016). Natural differences in isotopic abundance result from different assimilation rates which generate slight geographical variations in isotopic signature. These variations can then be used to trace migration or dispersal patterns. The approach relies on known and predictable variations in the isoscape or an isotope boundary (Hood-Nowotny & Knols 2007). For example, researchers recently found that the ^2H in the bodies of invasive Japanese beetles were closely correlated with ^2H in local precipitation across the USA and that their isotopic signatures changed predictably when exposed to simulated geographic changes in their water supply (Hungate *et al.* 2016). Others have used similar approaches to track discrete waves of invasions of Asian longhorned beetles (Heinrich & Collins 2016). These studies demonstrate the potential for tracking the timing of populations arriving at new locations and monitoring the efficacy of dispersal control and management measures in near-real-time.

Geographic variation in isotopes may elucidate invader dispersal over very long distances across variable isoscapes (such as human-mediated dispersal events that lead to introduction), but naturally occurring variation may be insufficient to unravel shorter distance dispersal events. Fortunately, high-resolution isoscape maps are becoming more widely available and, as more types of isotopes are surveyed [e.g., (Phillips & Gregg 2003; Phillips 2012; West, February & Bowen 2014)], the potential for addressing dispersal-related questions at smaller scales is increasing because a greater number of axes can be used to distinguish locations.

A key advantage of SIA in dispersal studies of animals is that it can be performed in a single capture event. In short, there is no need to catch, mark, release, and recapture specimens in the environment to estimate dispersal. This is crucial, because such experimental methods are often impractical or illegal for invasive species in their invaded range. In some cases, museum specimens may also be utilized if the preservation process does not introduce significant analytical bias. In fact, numerous studies have conducted SIA on tissues from museum/stored collections (Doucett, Giberson & Power 1999; Maguire & Grey 2006; Murphy, Bowman & Gagan 2007; Schmidt *et al.* 2007). For many taxa this is a key advantage, because it can allow easy access to a large number of specimens collected over long periods and from many localities [e.g., (Tillberg *et al.* 2007; Heinrich & Collins 2016; Hungate *et al.* 2016)].

The dispersal of many invasive animals may be difficult to study both in their native and invaded range due to their remote locations or cryptic behaviors. Conventional techniques [e.g., mark-recapture, telemetry, occurrence patterns, genetic studies (Ranius 2006)] are time consuming and costly methods of measuring the dispersal patterns of invasive species compared to SIA (Bodey, Bearhop & McDonald 2011). Moreover, in cases where ethical considerations prevent collection or euthanizing large numbers of animals, small subsamples of tissues can be used for SIA. Common examples include feathers in birds (Rubenstein & Hobson 2004; Prochazka *et al.* 2013; Pekarsky *et al.* 2015), fur or whiskers in large carnivores (Yeakel

et al. 2009; Voigt *et al.* 2014; Mutirwara, Radloff & Codron 2017), or baleen of whales (Schell, Saupe & Haubenstock 1989; Aguilar *et al.* 2014). As discussed below SIA can even be used to track dispersal patterns of plant seeds.

2.2 Artificial enrichment approaches

Another area where SIA can be employed to tackle dispersal-related questions in invasion ecology is through the use of *artificial enrichment* – a method that is perhaps especially well-suited to answer questions surrounding ‘fine-scale’ (e.g., < 1 km) dispersal of both plants and animals. Sessile, seed-dispersed plants represent a particularly challenging situation for estimating landscape-scale dispersal. To what extent do dispersal patterns differ between native and invasive species? Indirect approaches like genetic parentage or gene flow estimates are often used as surrogates to infer dispersal (Ouborg, Piquot & van Groenendael 1999). However, these are not only expensive, but also only provide crude dispersal estimates, since rare long-distance dispersal events often go undetected.

Artificial enrichment consists in integrating into the diet, or externally applying isotope-enriched compounds, which isotopic signature is different from the natural environment. This method has proven to be an efficient tool for tracking the dispersal of the invasive Amur honeysuckle shrub using topical (foliar) applications of ^{15}N -enriched urea ($\text{CH}_4\text{N}_2\text{O}$) to the maternal plant (Castellano & Gorchoy 2013). The authors assessed three concentrations of application across five time points (every three weeks) and then collected seeds and split these into those that were measured for ^{15}N and those allowed to germinate and grow under various conditions. Remarkably, even a single foliar application of ^{15}N was sufficient to effectively isotopically label both the seeds and the tissues of subsequent seedlings of this invasive shrub.

Other researchers have added ^{15}N -labeled ammonia (NH_4) to freshwater ponds and rivers inhabited by stonefly and mayfly larvae. The larvae consumed the ^{15}N -enriched detritus, resulting in adult flies that were isotopically distinct from natural populations but otherwise

identical (Fig. 1F). Traps placed around the ponds then allowed researchers to document the timing, distance, and direction of dispersal (Hershey & Pastor 1993; MacNeale, Peckarsky & Likens 2004; MacNeale, Peckarsky & Likens 2005). It has even been proposed that sanguivorous insect vectors (*e.g.*, mosquitos, tsetse flies, *etc.*) can be captured and fed isotopically enriched blood meals before being released to track dispersal, habitat range, *etc.* (Hood-Nowotny & Knols 2007). We know of no invasion studies that have employed this method in the field, most probably due to legal constraints when releasing the species in its invaded range.

Artificial enrichment can also be used to identify long-distance dispersal within the invaded range. Long-distance dispersal (*e.g.*, > 1 km) is a key process underlying some biological invasions, enabling rapid dispersal, fast population growth, and sampling of suitable habitat across the new range (Higgins & Richardson 1999). Identifying areas that are most likely to receive long distance-dispersed propagules is crucial for effective management (Wilson *et al.* 2009). A ¹⁵N-enrichment approach used to track long-distance seed dispersal of native populations of the European holly and hawthorn tree elegantly illustrates the context (habitat) dependency of dispersal events for these two species and showed the need to consider habitat heterogeneity to manage ecological connectivity of landscapes (Carlo *et al.* 2013).

3. DIET AND NICHE SPACE

Following introduction, the establishment and spread of a non-native species depends on it being able to extract sufficient nutrients from the environment to support its continual mass and energy demands associated with growth, survival, and reproduction. Understanding invader diets helps identify specific physiological traits that contribute to [or limit] invasion success. Such insights are needed to inform predictions about the impacts of invaders on native communities, and to identify potential management options. The ¹³C, ¹⁵N, and ³⁴S in an animal's tissues reflects that of its dietary resources – essentially “you are what you eat” [*sensu* (DeNiro &

Epstein 1976)]. SIA is, therefore, an ideal tool for characterizing dietary history. It has the added advantage that it provides longer-term estimates than acute behavioral observations or stomach content analyses, which only give 'snapshots' in time.

3.1 Dietary flexibility

To what extent are invasive species capable of tolerating changes in their diet? SIA has been used to show that invasive ants vary their diet across seasons, by rapidly exploiting periodic pulses in floral nectar availability (Mothapo & Wossler 2017). Another study found that introduced populations of the invasive carnivorous Argentine ant initially occupied a similar trophic level to native Argentinian populations (Tillberg *et al.* 2007). However, once established, the invasive ants shifted to a lower trophic position as they consumed a greater proportion of plant-based resources (*e.g.*, nectar and honeydew) following the drastic reductions in native Californian ant populations in the face of their invasion. Similar trophic flexibility was found in invasive red swamp crayfishes, where ^{13}C signatures revealed that they maintained a competitive advantage over native species during food shortages by increasing consumption of allochthonous riparian vegetation (Grey & Jackson 2012), and in invasive rats on Surprise Island in New Caledonia, that consumed seabird chicks when available, but switched to sea turtle hatchlings when seabirds were not present (Caut, Angulo & Courchamp 2008). Similar evidence from studies in aquatic environments indicates that invasive species often have a flexible and generalist diet [*e.g.*, (Zhang *et al.* 2010; Garton, Payne & Montoya 2011; Jackson *et al.* 2017b)]. Further isotope studies on invasive species across a variety of habitat types, and comparative studies between invasive and native congeners, are needed to determine the universality of such dietary flexibility and responses to resource availability among invasive species. SIA is particularly well-suited to detect subtle differences in resource use patterns, particularly in marine habitats where it is difficult to directly observe animals. For instance, SIA has been used to identify previously unknown feeding ecotypes within populations of amphipods

(Nyssen *et al.* 2005), delphinids (Kiszka *et al.* 2010), and penguins (Cherel & Hobson 2007). Such characteristics could even be altered at different stages of biological invasion (see below).

3.2 Trophic interactions between invaders and natives

In the last decade, ecologists have developed numerous stable isotope-derived metrics to quantify population and community trophic ecology. For instance, the metrics ‘Total Area’ (Layman *et al.* 2007) and ‘Standard Ellipse Area’ (Jackson *et al.* 2011) can be used to quantify the spread of individuals in isotopic space as a measure of niche width (Fig. 1G). Several studies have used these metrics in invasion ecology. For instance, researchers showed that invasive crayfish have a wider niche width than their native counterparts (Olsson *et al.* 2009). Another study reported changes in niche overlap between invasive and native fish (Hill *et al.* 2015). Isotopic niche space can also be detected through shifts in centroid location, or a change in variance (Bearhop *et al.* 2004; Turner, Collyer & Krabbenhoft 2010; Hammerschlag-Peyer *et al.* 2011; Guisan *et al.* 2014; Bond, Jardine & Hobson 2016).

Estimates of isotopic niche width across an invader’s range, and at different stages of invasion, can even be used to predict invader impacts (Comte, Cucherousset & Olden 2017), and to inform decisions about appropriate control or eradication plans. For example, lake productivity and size were shown to alter the trophic position and isotopic niche width of invasive red swamp crayfish (Jackson *et al.* 2017a). Studies of stable isotope niche space also show that invasions can have cascading effects, altering the niche width of other invaders or native species. For instance, eight years of isotope data during the establishment of invasive carp revealed that invasion by this fish caused a reduction in the dietary niche of a previously established invasive crayfish (Jackson *et al.* 2012).

3.3 Large-scale food web effects

Biological invasions inevitably cause shifts in food web structure since they automatically add a new 'node' (*i.e.*, the invasive species) and thus new 'links' [*i.e.*, their feeding interactions (Jackson *et al.* 2017b)]. These changes can alter ecosystems in several ways and across multiple levels of organization (from individuals to ecosystems), and multiple trophic levels (Cucherousset & Villegier 2015; Hette-Tronquart 2019). For instance, invasive predators can have conspicuous direct effects on their prey, with cascading consequences for lower trophic levels. Invaders may also have 'bottom-up effects' on the food web (*i.e.*, acting as a novel food source) or they may compete with native species for resources (Jackson *et al.* 2017b). Researchers gathered 4030 isotopic values for fish from 496 communities to assess invasive fish impacts and found that the presence of invasive species invariably alters isotopic community structure, but that the effects differed across ecosystem type (Sagouis *et al.* 2015). For example, in running waters, the non-natives (primarily predators) increased the total community isotopic niche without altering the niche breadths of native fish species. In still waters, non-natives modified basal resource consumption and reduced the isotopic niche breadths of natives through competition.

In one of the first applications of SIA in invasion ecology researchers showed that invasive smallmouth bass and rock bass caused a shift in trophic position and dietary habitat use of native lake trout (Vander Zanden, Casselman & Rasmussen 1999). Another study indicated that zooplankton gradually became more reliant upon terrestrial detritus when filter feeding zebra mussels invaded a lake in Ireland where they competed for algal resources (Maguire & Grey 2006). SIA has since been used to demonstrate that the ecological effects of invasions can cascade across ecosystem boundaries. For instance, a study of ^{13}C and ^{15}N revealed the far-reaching effects of invasive fish on the diets of terrestrial spiders (Jackson *et al.* 2016b). Another study used measures of ^{15}N to show that seabirds enhance coral reef productivity in the absence of invasive rats on islands (Tabak *et al.* 2016). Soils and coastal shrubs on rat-free islands were highly enriched in ^{15}N compared to rat-infested islands,

reflecting pelagic nutrients transferred by seabirds. This higher $\delta^{15}\text{N}$ was also found in microalgae, sponges, and herbivorous damselfish on adjacent coral reefs. Researchers recently demonstrated that co-invasion of a bivalve with invasive corals does not involve nutritional links, but rather that the invasive coral creates habitat that facilitates co-invasion of the mussel (Vinagre *et al.* 2018). Additional examples of this phenomenon known as ‘invasional meltdown’ are discussed in Section 5.

3.4 Considerations for diet analyses

The pressures that drive changes in niche space can differ with ontogeny and may occur at different times within the lifetime of long-lived species (Cherel *et al.* 2009; Hammerschlag-Peyer *et al.* 2011). It is therefore important to measure tissues whose elemental turnover rate reflects the period of interest [e.g., (Ayliffe *et al.* 2004; Bauchinger & McWilliams 2009; Church *et al.* 2009; Vander Zanden *et al.* 2015)]. For example, blood plasma or liver tissues tend to reflect recently consumed foods whereas muscle or connective tissues tend to integrate dietary history over longer periods (Tieszen *et al.* 1983; Schell, Saupe & Haubenstock 1989). Researchers showed that after a diet shift the $\delta^{13}\text{C}$ of metabolically active tissues (e.g., body fat or reproductive organs) changed faster than more metabolically inert tissues, and that the speed of change can differ between sexes (Gratton & Forbes 2006). In the Japanese beetle study described above, researchers noted that although the isotope signatures of their whole bodies changed those of the wing and elytra tissues remained constant (Hungate *et al.* 2016). Such tissue-level differences are fortuitous because they reflect the isotope signatures of both the larval conditions and that of the new habitat.

Besides depicting the diet and niche space of invasive species, SIA can also be used to detect whether native species use invasive species as food resources in different regions, populations, and across time. Some animals such as tortoises are unique in that they ‘record and carry’ decades of isotopic data in their scutes (Fig. 1H). By measuring ^{13}C and ^{15}N in

keratinized growth rings of tortoise shells researchers showed that individuals used plant resources very differently across years (Murray & Wolf 2013). For example, some individuals in drier areas showed episodic shifts between diets that incorporated plants with C_3 or C_4 /CAM photosynthetic pathways while other individuals in wetter areas had a more constant diet with higher reliance on C_3 plants across their lifetime. It is thus possible to detect dietary shifts and to reconstruct diet history or seasonality of tortoises inhabiting areas with invasive plants or along a plant invasion gradient. Despite the potential of this approach, the isotopic values of growth rings are not purely discrete and carbon from a new diet can be incorporated into older rings (“carbon creep”) – especially those adjacent to the new rings formed after the diet switch (Murray & Wolf 2012). Therefore, this methodology requires calibration and cautious interpretation of isotope chronologies in addition to species-specific parameter estimates given the variation in different tissues’ turnover rates and discrimination values among reptiles (Reich, Bjorndal & Martinez Del Rio 2008; Rosenblatt & Heithaus 2013). Overall, the use of SIA to monitor dietary changes over long time spans should be further explored to quantify how invasion-induced niche shifts are correlated with other variables such as the warming climate and ongoing habitat loss thereby providing insight into how these multiple stressors interact.

4. PHYSIOLOGICAL DIFFERENCES BETWEEN INVADERS AND NATIVE ANIMALS

4.1 Assimilation efficiency

Animals are unable to absorb all the nutrients they ingest. Invasive species that can maximize nutrient uptake may have an advantage over others, possibly increasing their potential success. Overall, differences in dietary nutrient assimilation could have profound effects on the survival and persistence of an invader and it is possible to compare assimilation efficiencies between native and invasive animals. We discuss water uptake and nutrient assimilation of plants below, but the following questions generally apply to both groups. Do successful invaders have higher dietary assimilation efficiencies? If so, does this pattern apply

to all classes of macronutrients equally? SIA offers an opportunity to answer these physiological questions.

Traditionally, dietary assimilation efficiency of animals has been studied by calculating differences between gross energy intake and the energetic content of excreta (e.g., feces and urates). SIA can expand existing approaches by further allowing researchers to quantify digestive assimilation efficiency of different diets and even different nutrients. In practice this is done by feeding animals meals (supplemented with specific ^{13}C - or ^{15}N -labeled molecules) and then measuring the amount of the tracer recovered in the animals' wastes (e.g., breath, feces, urates, etc.; Fig. 1A). For instance, sphinx moths (Levin, McCue & Davidowitz 2017) and house sparrows (McCue *et al.* 2011) had far higher assimilation efficiencies of dietary amino acids than fatty acids (Levin, McCue & Davidowitz 2017). Similarly, a study with humans used this approach to show that cooked egg proteins had much higher assimilation efficiencies than raw egg proteins (Evenepoel *et al.* 1999).

The specific type of tracer molecules used can vary depending on the type of macromolecules (e.g., carbohydrates, lipids, or proteins) thought to be limiting in the diet. The choice of isotope tracer (e.g., ^{13}C or ^{15}N) used in feeding studies can also provide unique perspectives into nutrient dynamics. ^{13}C -labeled dietary tracers provide a good proxy of bulk energy assimilation for a given class of macronutrients. They also have the advantage of providing simultaneous information about the rates of oxidation for dietary nutrients if combined with ^{13}C -breath testing (see below). For example studies of reptiles showed that postprandial lizards (Plasman *et al.* 2019) and pythons (McCue, Passement & Guzman 2015) oxidized a much greater proportion of dietary proteins than lipids.

^{15}N -labeled tracer molecules (e.g., amino acids) provide an excellent proxy of protein assimilation because nitrogen is generally absent from lipids and carbohydrates. Although the ^{15}N tracer cannot be detected in the exhaled breath, it can be readily measured in the feces and urates [e.g., (Hawkins 1985; Carter *et al.* 1994; Sponheimer *et al.* 2003; Balter *et al.* 2006)].

Moreover, because nitrogen is far less abundant in the diet than carbon, a given dose of ^{15}N -labeled amino acid will give a stronger isotopic 'signal' than an equivalent, singly- ^{13}C -labeled amino acid (Gaudichon *et al.* 1999; Durso & French 2017).

4.2 Energy use during resource limitation

Invasive species establishing new populations may experience forms of food or water stresses that may differ from what they have faced in their historical evolutionary contexts. In new habitats these stressors could also occur at different frequencies or have different durations compared to their native range. One of the most commonly documented organismal responses to food or water stress is to behaviorally reduce activity and physiological functionality to minimize metabolic rates as occurs during hibernation, estivation, dormancy, diapause, or starvation-induced hypometabolism (Storey & Storey 1990; Geiser 2004; McCue 2010; McKechnie & Mzilikazi 2011; Hohtola 2012; McAllen & Geiser 2014; Ruf & Geiser 2014); but no animals can completely eliminate these costs.

Researchers can isotopically label discrete carbon pools in the body of animals (e.g., by repeatedly feeding them with meals artificially enriched with ^{13}C -labeled lipids or proteins) and conducting ^{13}C -breath testing (*i.e.*, measurements of $^{13}\text{CO}_2$ release in the exhaled breath) to explore how they alter their reliance on their different types of metabolic fuels (Welch Jr *et al.* 2016). Ideally, these controlled feedings may occur over the course of an animal's life (McCue *et al.* 2013a; McCue *et al.* 2015), but should at least include a biologically relevant period (e.g., hours and days for plasma and liver versus weeks and months for muscle and connective tissue) that is sufficient to permit measurable enrichment of endogenous tissues (Munoz-Garcia *et al.* 2012; McCue *et al.* 2013b; Khalilieh, McCue & Pinshow 2015).

In practice, diets that are supplemented with certain ^{13}C -labeled amino acids will gradually enrich the body proteins. Essential amino acids are preferred for this purpose because they are less likely than non-essential amino acids to be immediately oxidized upon ingestion

(Berthold *et al.* 1991; McCue *et al.* 2010). Diets supplemented with ^{13}C -labeled fatty acids will isotopically enrich the body lipids. Essential fatty acids (e.g., arachidonic acid, linolenic acid, *etc.*) are not particularly useful for this approach because they can be prohibitively expensive and only account for a small proportion of the total body lipids [e.g., (Holmstrup, Hedlund & Boriss 2002; McCue 2008; Ben-Hamo *et al.* 2013; Mustonen *et al.* 2013)]. Common non-essential fatty acids (e.g., oleic acid, palmitic acid) are sufficient to cause measurable enrichment of the body lipids over time. ^{13}C -Glucose has been used to enrich hepatic and/or muscular glycogen stores (Gay *et al.* 1994a; Gay *et al.* 1994b; Muller *et al.* 1997; Tanis *et al.* 1998; Tanis *et al.* 2003). However, because animals can readily interconvert the carbon atoms of dietary glucose into both non-essential amino and fatty acids, it can be problematic to quantitatively interpret the experimental outcomes (McCue 2013).

Otherwise identical populations of these artificially ^{13}C -protein-enriched or ^{13}C -lipid-enriched animals can then be exposed to resource (e.g., food or water) limitation and changes in their rates of endogenous nutrient oxidation can be compared over time within and across species (Fig. 1D). This experimental approach has revealed that insects from different taxonomic orders vary widely in how they mobilize their finite lipid and protein resources (McCue *et al.* 2015). This approach has also been used to show that quail starving for several days oxidize stored lipids at a relatively constant rate yet exhibit large diel fluctuations in their rates of protein oxidation – a pattern that markedly differs from the typical mammalian response (McCue *et al.* 2013a). We are not aware of this experimental approach being used yet to explore the fundamental rules that govern resource use between competing native and invasive animal species, but studies using artificially enriched tracers in plants have revealed important physiological differences during resource limitation (see below).

4.3 Reproduction

Survival alone does not ensure the successful establishment of an invasive species; invaders also need to reproduce. It has been suggested that some invasive species benefit from allocating a greater proportion of their energy resources towards reproduction (Morris, Walck & Hidayati 2002; Lambrecht-McDowell & Radosevich 2005; Mason *et al.* 2008). SIA can be used to measure these vertical transfers (*i.e.*, from parents to offspring) of nutrients (Fig. 1B). In fact, previous studies of animals switched between C₃- and C₄-plant-based diets have permitted researchers to observe the vertical transfer of bulk nutrients into eggs (O'Brien, Schrag & Martinez del Rio 2000; O'Brien, Fogel & Boggs 2002) and milk (Chevalier, Pelletier & Gagnon 1984; Boutton *et al.* 1988; Metges, Kempe & Schmidt 1990; Doronin *et al.* 2012). We are unaware of studies comparing patterns of resource allocation towards reproduction between native and invasive animals.

SIA can be used to reveal where an animal exists along the continuum of capital-income breeding strategies (Inger & Bearhop 2008; Van Dyke & Beaupre 2012; Boggs & Niitepold 2014). In practice, this involves providing isotopically labeled nutrients to a parental generation and recovering the tracers in the eggs or offspring. ¹⁵N-tracers fed to several species of snakes during vitellogenesis showed that they varied widely in their reliance on income resources (Van Dyke, Beaupre & Kreider 2012). This experimental approach also revealed that flesh flies used stored (*i.e.*, capital) resources for their first clutches of eggs but used recently ingested (*i.e.*, income) resources for their second clutch (Wessels, Jordan & Hahn 2010); no such differences were observed in lizards (Warner *et al.* 2008). In related tests of nutrient transfer ¹⁵N-labeled aphids were fed to predatory insects and spiders (Nienstedt & Poehling 2000; Nienstedt & Poehling 2004).

If individual classes of macromolecules are isotopically labeled, then researchers can make even finer distinctions about the vertical transfer of nutrients. Two studies used this approach to demonstrate that both lizards (Warner *et al.* 2008) and ducks (De Vink *et al.* 2011) provisioned eggs with proteins from recently ingested meals. In contrast, the egg lipids were

derived exclusively from stored resources. Another study documented tradeoffs between allocation of dietary proteins to reproduction and wound-healing in lizards (Durso & French 2017). Native gray-headed sparrows infected with malaria showed a similar trade-off in protein allocation, but invasive house sparrows showed no such pattern (Coon *et al.* 2014).

5. PLANTS AND SOILS

The success of many plant invaders hinges on their capacity to extract resources from the environment at greater rates or efficiencies than native species. Such resources are then allocated to structures (*e.g.*, seeds) or processes (*e.g.*, prolonged flowering), which likely further improve the success of the invasive plants. In nutrient-limited ecosystems, even small differences in efficiency of resource acquisition may become important, owing to the energy and carbon costs involved under these conditions.

5.1 Water sourcing and water use efficiency

Water is the most limiting non-mineral resource for terrestrial plant distribution, but its abundance can vary across time and space. SIA can also be used to determine invasive plants' water use efficiencies (Seibt *et al.* 2008) and explore the long-term behaviors of plant stomata during seasonal cycles. This method relies on the fact that the major CO₂-fixing enzyme for photosynthesis, Rubisco, has a higher affinity for the more abundant ¹²CO₂ molecule, compared to the scarcer, less common ¹³CO₂. During water limitation, there is usually an associated reduction in stomatal conductance. Consequently, the internal, sub-stomatal CO₂ concentration becomes reduced in leaves. Since Rubisco would first favor the fixation of the lighter ¹²CO₂ molecules, the ¹²C:¹³C ratio gradually declines owing to less discrimination against the available ¹³CO₂.

The lower discrimination values are driven by stomatal closure and/or a higher photosynthetic water use efficiency (WUE) (Richards 1996; Werner *et al.* 2012). Measures of WUE indicate

491 how much CO₂ is fixed per unit of water lost via the leaf stomata. Using $\delta^{13}\text{C}$ allows comparison
492 of water relations in invasive and native plants over long periods or during a specific growing
493 season (Fig. 1E). This would indicate the capacity of invasive plants to acquire and use water
494 efficiently, and can provide further insights into the ability of the invasive species to survive
495 water limitations (Werner *et al.* 2012).

496 Stratton and Goldstein (2001) cautioned that care should be taken when using ^{13}C
497 values as indicators of long-term or integrated WUE, because the ^{13}C values may be incorrectly
498 interpreted when the species' leaf production is not taken into account. In their study, the ^{13}C
499 values of the invasive Brazilian peppertree and six native Hawaiian tree species showed that
500 the rapid growth of the invasive tree is due to its greater ability to deal with variation in water
501 availability, but that the seasonal leaf expansion of the species greatly affected the ^{13}C values
502 (Stratton & Goldstein 2001).

503 Researchers have used $\delta^{13}\text{C}$ values to compare WUE over two seasons between
504 invasive gamba grass (*Andropogon gayanus*) and a native grass in Australia. Both C₄ grasses
505 had similar WUE during the wet rainy season. However, during the dry season the native grass
506 senesces while gamba grass showed higher WUE compared to the wet season (Ens *et al.*
507 2015). This physiological plasticity has probably contributed to the invasiveness of gamba grass
508 in Australia.

509 Invasive plants often have major impacts on water resources throughout an ecosystem
510 and SIA can be used to investigate these (Antunes *et al.* 2017). Researchers measured the ^{18}O
511 in xylem water and used a mixing model to partition the contribution of precipitation versus
512 ground water in invasive acacia in Europe and showed that the species exhibits large
513 seasonally plastic responses (Maguas *et al.* 2011). A similar approach was used to document
514 water sourcing in invasive trees in Sudan and USA (Saito *et al.* 2014). Both studies suggested
515 that the increased capacity for the invasive to adapt to changing water sources may provide
516 competitive advantage over natives.

5.2 Soil carbon and microbial communities

SIA has utility in elucidating trophic relationships in hidden biodiversity compartments, where other approaches are difficult to apply. For example, in the past two decades SIA has revolutionized our understanding of soil food webs which are critical to the functioning of terrestrial ecosystems (Potapov, Tiunov & Scheu 2018). It was revealed that these webs are based primarily on microbial resources and not dead plant material, and that different taxonomic groups of microbes are linked into different pools of organic carbon in the soil. For example, an invasive Australian acacia was recently shown to decrease the ^{13}C of organic matter in the soil (Ulm *et al.* 2017), but the role of microbes in this response is not fully understood.

The potentially profound impacts of below- and aboveground invasions on soil communities has been studied using SIA. In an interesting application of SIA to below-ground dynamics, researchers showed that the noxious spotted knapweed parasitizes carbon from native grass species via belowground mycorrhizal networks. This mechanism may contribute to the wholesale competitive exclusion of natives, a common feature of perennial plant invasions (Carey, Marler & Calloay 2004).

Invasive species, especially plants, can have pronounced impacts on the microbial components of the environments they invade. Dense stands of invasive plants can alter soil nutrient loads through root exudates and inputs via leaf litter which in turn affect soil nutrient status and the functionality, diversity and structure of soil microbial communities (Waring *et al.* 2015; Hu *et al.* 2018). These soil microbial communities play an important role in the biogeochemical cycling of soil elements; invader-induced changes in the abundance and composition of microbial communities thus represent a significant ecological impact [*e.g.*, (Yang *et al.* 2016)]. Despite their importance and vast diversity, the taxonomic identity of microbes involved in specific processes has historically been confined to the small fraction of culturable organisms. While this has changed with the advent of high-throughput next-generation

sequencing (NGS) approaches, comparable advances in determining microbial functionality is still lagging. SIA may provide opportunities to overcome this limitation.

Stable-isotope probing (SIP) is a technique developed to trace nutrient fluxes during biogeochemical cycling by microbes. When microbial communities are exposed to (fed) substrates enriched with heavier stable isotopes those taxa that incorporate them can be separated from those containing lighter isotopes through isopycnic centrifugation (Radajewski *et al.* 2000). DNA isolated from the heavier isotope of microorganisms can then be characterized taxonomically using traditional NGS analysis (e.g., 16S rDNA gene metabarcoding). In practice, SIP has been used to show that only a subset of rhizosphere microbiomes metabolize photosynthetically-derived carbon in various crop plants (Haichar *et al.* 2008). This was done by allowing plants to fix artificially enriched $^{13}\text{CO}_2$ into their tissues, followed by tracking of $^{13}\text{CO}_2$ into soil microbial biomass (Haichar *et al.* 2008). Using a similar approach researchers treated coastal mudflats with ^{13}C -labeled methane and determined that net methanotrophic activities were attenuated in areas where alien plants were present (Deng *et al.* 2019). SIP may hold promising future research directions in invasion science. For example, providing microbial communities of neighboring invaded and uninvaded habitats with isotope-enriched substrates may not only provide key insights on how invasive species impact microbial community diversity but also function.

5.3 Nitrogen fixation and nitrogen uptake

The use of SIA is not limited to water and carbon relations and can also be applied to the acquisition of nitrogen, especially by invasive legumes which indirectly fix atmospheric N_2 (Werner *et al.* 2010; Ulm *et al.* 2016). Invasive plants in nutrient-limited ecosystems are often legumes (Paynter *et al.* 2003). Most legumes form symbioses with bacteria, known as rhizobia, which can acquire N_2 gas from the atmosphere via biological nitrogen fixation (BNF). Rhizobia fix nitrogen through nitrogenase activity that reduces atmospheric N_2 to ammonia. Nitrogenase

preferentially incorporates the lighter isotopes of atmospheric N₂ (Unkovich 2013). Measures of $\delta^{15}\text{N}$ can therefore be used to determine the contribution of BNF to the N budget of these invasive legumes (Fig. 1C). With careful experimental planning and analyses, the efficiency of BNF can be calculated for these legumes in nutrient-limited soils (Magadlela *et al.* 2016; Magadlela *et al.* 2017).

It is of great importance to the survival of the invasive plants, not only to be able to acquire N from both soil and atmospheric source, but to do so efficiently. For example, across 19 non-native Australian *acacia* species with varying levels of invasiveness in nutrient-poor soils of South Africa's fynbos vegetation, invasion success (in terms of geographic spread of invasive populations) was unrelated to N-fixation efficiency (determined using $\delta^{15}\text{N}$ signatures) as all species fixed N equally efficiently (Keet *et al.* 2017). In other studies of N-uptake researchers injected the soil with a $^{15}\text{NH}_4$ tracer to investigate intraspecific and interspecific competition and determined that invasive acacias somehow reduced the ability of native plants to take up nitrogen (Werner *et al.* 2010) or otherwise outcompeted the native plants for available nitrogen (Peperkorn, Werner & Beyschlag 2005).

Over time, invasive legumes tend to enrich soils with nutrients, especially nitrogen. This process may lead to indirect impacts on above- and belowground soil communities that resemble 'small-scale' isoscapes (Rascher *et al.* 2012). Again, SIA provides unique opportunities to investigate such local impacts. For example, researchers have examined the impacts of invasive long-leaved wattle in dune systems in Portugal (Hellmann, Werner & Oldeland 2016). Using foliar $\delta^{15}\text{N}$ isoscapes together with nitrogen concentrations of co-occurring native species, they found the native species to have a more than twofold increase in foliar N and a $\delta^{15}\text{N}$ enrichment of up to 8‰, when growing near the invasive species. Depending on the native species the foliar ^{15}N values can either be increased or decreased by the presence of long-leaved wattle (Hellmann, Werner & Oldeland 2016).

SIA can also help researches determine the spatial scales of ecosystem invasion impacts, such as altered nitrogen cycling. Community-scale native foliage $\delta^{15}\text{N}$ isoscapes have, for example, shown that the spatial dimension of changes associated with the invasion of an exotic N_2 -fixing acacia species was much greater than the distribution of the invasive species itself (Rascher *et al.* 2012).

5.4 Invasional meltdown

Invasive species can have profound impacts on critical ecosystem processes, such as carbon and nutrient cycling (D'Antonio & Vitousek 1992; Simberloff 2006). The system changes they drive can in turn favor their competitive dominance and spread via self-reinforcing positive feedback loops that might be a ubiquitous feature of successful invasions (Levine *et al.* 2006; Le Roux *et al.* 2017). Also of concern is the possibility that the impacts of one invasive species might increase the susceptibility of an ecosystem to further invasions, a phenomenon known as *invasional meltdown* (Simberloff & Von Holle 1999). Invaders can facilitate subsequent invasions through several pathways, including impacts on ecosystem properties, direct nutritional links, or habitat creation. SIA can be used to understand these pathways to invasional meltdown.

Invasion of Hawaiian woodlands by C_4 grasses has altered community composition and important ecosystem drivers, such as fire cycles, N-mineralization, and net primary productivity (Mack, D'Antonio & Ley 2001). Dominance of one invasive grass in the years after fire dramatically increased N-cycling rates. Increased N availability favored the N-limited invasive grass in a positive feedback loop further reinforcing its dominance. However, researchers found that this feedback loop has not persisted through time (Yelenik & D'Antonio 2013). They used SIA to show that later-stage invaded soils have suffered loss of nitrogen through denitrification pathways and that long-term ecosystem N-depletion feeds back negatively on the dominance of invasive C_4 grasses.

Alteration of ecosystem process such as nitrogen cycling can result in invasional meltdown if impacts of one invasive species facilitates invasion by others. Ecosystem state shifts are likely to persist after eradication of invaders (so-called *legacy effects*) and complicate the process of restoring native communities at previously invaded (Yelenik & D'Antonio 2013) or disturbed sites (Rascher *et al.* 2011). In the C₄ grass example from Hawaii discussed above, for instance, the long-term ecosystem N-depletion under invasion does not favor native species re-establishment, but rather benefits invasive N-fixing trees (Yelenik & D'Antonio 2013).

6. PATHS FORWARD

Despite the popularity of SIA in specific areas of ecological research, its application in addressing pertinent questions in invasion biology has only recently emerged. What makes some species successful invaders while others fail is a pivotal question that is attracting major research effort [e.g., (Fournier *et al.* 2019)]. Despite major advances, much uncertainty remains, largely due to the high level of context dependency of invasions (*i.e.*, introduction history (timing and pathways) and attributes of both invasive species and the invaded habitat must be considered) (Richardson & Pysek 2006; Thuiller *et al.* 2006). This review makes it clear that SIA presents biologists with unprecedented and exciting opportunities to better understand many of the key processes and mechanisms that underlie successful biological invasions.

A recent horizon scanning analysis identified emerging scientific, technological, and sociopolitical issues that are likely to affect how biological invasions will be studied and managed over the next two decades (Ricciardi *et al.* 2017). SIA has great potential to contribute insights in most of the broad categories of topics identified in this exercise. For example, within the theme of biotechnological issues, changing agricultural practices were identified as a potential driver of major changes in the trajectories of invasions. New investments by commercial businesses are seeking to enhance production through the more effective use of

soil bacteria and fungi. Such practices could trigger invasions by currently non-invasive crops or co-occurring plants but may also have substantial impacts on soil microbiomes. While new insights are emerging on the effects that some invasive plants have on soil microbiomes, our understanding of their magnitude is limited by the approaches available to evaluate them. Next-generation sequencing (NGS) has increased our capacity to understand soil diversity impacts [e.g., (Kamutando *et al.* 2017)], but often this approach lacks functionality [but see e.g., (Jackson *et al.* 2016a)]. Even diversity metrics derived from NGS are largely uninformative due to low taxonomic resolution. Although SIA cannot circumvent this issue, it can provide more precise information about functional changes in microbiomes, for example, using stable-isotope probing (SIP) approaches. Combining NGS, SIP, and soil microbial enzymatic analyses is a promising approach to better understand invader impacts on microbiomes.

Half of the emerging challenges and opportunities in invasion science identified by Ricciardi *et al.* (2017) were categorized as “sociopolitical issues”; these are areas where human perceptions and values either complicate management options or potentially provide new opportunities for dealing with biological invasions. There are also clear opportunities for SIA to address key questions in such research areas. Conflicts of interest that thwart effective management of invasions often revolve around imperfect understanding of the impacts of invasive species (Woodford *et al.* 2016). A striking example is the debate around the impact of non-native trout in South Africa, where parties are calling for trout to be removed from the list of invasive species in national regulations. These parties argue that the impact of trout on native biodiversity is unsubstantiated and emphasize that many people derive pleasure and, in some cases, income from opportunities that trout provide for recreational fishing. Such conflicts of interest greatly complicate the management of this species in South Africa and of many other invasive species worldwide (Woodford *et al.* 2016).

Many alien plants that were originally introduced to provide new ecosystem services or to supplement existing ecosystem services have become invasive. Conflicts of interest relating

to the management of such species usually relate to perceptions and assumptions about various facets of resource use by the alien plant. Many studies have used SIA to characterize water use of alien trees in arid ecosystems and riparian habitats and to clarify the extent to which the alien species compete with native species for water [e.g., (Busch & Smith 1995)]. Isotope studies have been used to provide motivation for expensive management interventions – e.g., in the case of *Prosopis* invasions in the Northern Cape of South Africa where invasive stands use large volumes of groundwater (Dzikiti *et al.* 2013).

A clear objective of much research in invasion science is to inform efforts to restore habitats impacted by invasive species [e.g., (Holmes *et al.* 2005)]. The examples above demonstrate that SIA should be an important part of the toolbox required to understand the diverse impacts of invasions on, for example, food web structure and ecosystem functioning, but isotopes also have potential as a tool for monitoring the success of restoration efforts. For example, in conjunction with more traditional diversity-based approaches, SIA has been used to confirm that saltmarsh restoration attempts have been successful in re-establishing functioning native communities with food web structure and carbon cycling dynamics that closely approximate those of pre-invasion communities (Kindscher & Tieszen 1998; Dibble, Pooler & Meyerson 2013). Analyzing isotopic signatures at different depths along soil cores potentially provides a mean of temporally tracking ecosystem functions, such as carbon cycling, through the invasion-restoration trajectory, and for establishing pre-invasion baselines (Kindscher & Tieszen 1998).

In conclusion, we propose that several concurrent, innovative advances in tools and techniques (e.g., trophic mixing models), increasing availability of high-resolution isotope landscape maps for diverse stable isotopes, and reduced costs and an expanded range of artificial chemical enrichment products, are allowing key questions in invasion ecology to be tackled with unprecedented resolution and precision. Tackling classic problems in ecology using both laboratory and field investigations with these promises to provide insights to determine the

fundamental mechanisms and impacts that invasive organisms may have on native species communities.

ACKNOWLEDGEMENTS

This article arose from a workshop on isotopes in invasion biology funded by the DST-NRF Centre of Excellence for Invasion Biology (CIB). MDM was partly supported by a CIB visiting scientist fellowship. We are grateful for comments from the anonymous referees that helped improve the work.

AUTHOR'S CONTRIBUTIONS

MDM, SCT and JST conceived project; All authors contributed to ideas, data collection and literature review; MDM led the writing; MDM and JJLR developed the graphical presentation; All authors contributed to writing of sections, edited and approved the final ms.

DATA AVAILABILITY

There are no raw data for this paper.

Box 1. Fractionation

Fractionation is the result of physicochemical forces that cause differential abundances of heavy and light isotopamers throughout the environment (*i.e.*, isoscape) and within the organism which allows ecologists to make predictions about where an animal has been, or what it is eating. Some common variations in isotope signatures are:

- Naturally occurring differences in H and O isotopic signatures in the environment are primarily the result of equilibrium fractionation (*e.g.*, phase changes of water) (IAEA 2000; Fry 2006; Werner *et al.* 2012). H signatures might also present information about trophic status within a given food chain (Vander Zanden *et al.* 2016).
- Plants exploiting C₃ and C₄ photosynthetic pathways exhibit kinetic fractionation (*e.g.*, during biochemical reactions) and differentially fractionate against CO₂ molecules with different isotopic composition [*e.g.*, ¹³CO₂ and ¹²CO₂] (Whelan, Sackett & Benedict 1973; O'Leary 1988; O'Leary, Madhavan & Paneth 1992). In short, C₃ plants discriminate more against ¹³CO₂ and thus contain measurably lower levels of ¹³C in their tissues.
- Marine-derived carbon is isotopically variable (Farquhar, Ehleringer & Hubick 1989), but tends to contain ¹³C levels that are higher than terrestrial-derived carbon (Chisholm, Nelson & Schwarcz 1982; Rubenstein & Hobson 2004; Inger & Bearhop 2008; Quillfeldt *et al.* 2008; Schimmelmann 2011).
- Nitrogen accounts for approximately 6% of the mass of all proteins. The most commonly studied fractionation pattern is trophic enrichment, where the proteins in heterotrophs become more enriched than the protein they consume in their diets due to preferential retention of the heavy isotopamer during tissue renewal (Hobson *et al.* 1996; Kelly 2000; Post 2002; Caut, Angulo & Courchamp 2009).
- ¹⁵N levels in marine ecosystems also tend to be higher than terrestrial ecosystems (Coplen *et al.* 2002; Evans Ogden, Hobson & Lank 2004; Grey, Waldron & Hutchinson 2004; Nardoto *et al.* 2006).
- Nitrogen-fixing plants, mostly legumes, typically have lower levels of ¹⁵N since the enzyme responsible for biological N-fixation by rhizobia, nitrogenase, preferentially incorporates the lighter isotope of atmospheric N₂ gas (Unkovich 2013). Other invasive legumes including *Acacia* spp. may have ¹⁵N levels in leaf tissues that are higher than native species (Rascher *et al.* 2012).

7. REFERENCES

- Aguilar, A., Gimenez, J., Gomez-Campos, E., Cardona, L. & Borrell, A. (2014) $\delta^{15}\text{N}$ Value does not reflect fasting in Mysticetes. *PlosOne*, **9**, e92288.
- Allendorf, F.W. & Lundquist, L.L. (2003) Society for Conservation Biology Introduction: Population Biology, Evolution, and Control of Invasive Species. *Conservation Biology*, **17**, 24-30.
- Antunes, C., Diaz-Barradas, M.C., Zunzunegui, M., Vieira, S. & Maguas, C. (2017) Water source partitioning among plant functional types in a semi-arid dune ecosystem. *Journal of Vegetation Science*, **29**, 671-683.
- Ayliffe, L.K., Cerling, T.E., Robinson, T., West, A.G., Sponheimer, M., Passey, B.H., Hammer, J., Roeder, B., Dearing, M.D. & Ehleringer, J.R. (2004) Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed on an isotopically varied diet. *Oecologia*, **139**, 11-22.
- Balter, V., Simon, L., Fouillet, H. & Lecuyer, C. (2006) Box-modeling of $^{15}\text{N}/^{14}\text{N}$ in mammals. *Oecologia*, **147**, 212-222.
- Bauchinger, U. & McWilliams, S.R. (2009) Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. *Physiological and Biochemical Zoology*, **82**, 787-797.
- Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & Macleod, H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, **73**, 1007-1012.
- Bellard, C., Cassey, P. & Blackburn, T.M. (2016) Alien species as a driver of recent extinctions. *Biology Letters*, **12**, 20150623.
- Ben-Hamo, M., McCue, M.D., Khozin-Goldberg, I., McWilliams, S.R. & Pinshow, B. (2013) Ambient temperature and nutritional stress influence fatty acid composition of structural and fuel lipids in Japanese quail (*Coturnix japonica*) tissues. *Comp Biochem Physiol* **166A**, 244-250.
- Berthold, H.K., Hatchey, D.L., Reeds, P.J., Thomas, O.P., Hoeksema, S. & Klein, P.D. (1991) Uniformly ^{13}C -labeled algal protein used to determine amino acid essentiality *in vivo*. *Proc Natl Acad Sci*, **88**, 8091-8095.
- Blackburn, T.M., Pysek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarosik, V., Wilson, J.R.U. & Richardson, D.M. (2011) A proposed unified framework for biological invasions. *Trends Ecol Evol*, **26**, 333-339.
- Bodey, T.W., Bearhop, S. & McDonald, R.A. (2011) Invasions and stable isotope analysis – informing ecology and management. *Island invasives: eradication and management* (eds C.R. Veitch, M.N. Clout & D.R. Town), pp. 148-151. IUCN, Gland, Switzerland.
- Boggs, C.L. & Nitepold, K. (2014) Insights from stable isotope tracers on reproductive allocation under stress. *Integrative and Comparative Biology*, **54**, 880-889.
- Bond, A.L., Jardine, T.J. & Hobson, K.A. (2016) Multi-tissue stable-isotope analyses can identify dietary specialization. *Methods in Ecology and Evolution*.
- Boutton, T.W., Tyrrell, H.F., Patterson, B.W., Varga, G.A. & Klein, P.D. (1988) Carbon kinetics of milk formation in holstein cows in late lactation. *Journal of Animal Science*, **66**, 2636-2645.
- Bowen, G.J., Liu, Z., Vander Zanden, H.B., Zhao, L. & Takahashi, G. (2014) Geographic assignment with stable isotopes in IsoMAP. *Methods in Ecology and Evolution*, **5**, 201-206.
- Braden, B., Lembcke, B. & Caspary, W.F. (2007) ^{13}C -breath tests: current state of the art and future directions. *Digestive and Liver Disease*, **39**, 795-805.
- Busch, D.E. & Smith, S.D. (1995) Mechanisms associated with decline of woody species in riparian ecosystems of the southwestern U.S. *Ecological Monographs*, **65**, 347-370.
- Carey, E.V., Marler, M.J. & Calloay, R.M. (2004) Mycorrhizae transfer carbon from a native grass to an invasive weed: evidence from stable isotopes and physiology. *Plant Ecology*, **172**, 133-141.
- Carlo, T.A., Garcia, D., Martinez, D., Gleditsch, J.M. & Morales, J.M. (2013) Where do seeds go when they go far? Distance and directionality of avian seed dispersal in heterogeneous landscapes. *Ecology*, **94**, 301-307.

- Carter, C., Owen, S., He, Z., Watt, P., Scrimgeour, C., Houlihan, D. & Rennie, M. (1994) Determination of protein synthesis in rainbow trout, *Oncorhynchus mykiss*, using a stable isotope. *Journal of Experimental Biology*, **189**, 279-284.
- Castellano, S.M. & Gorchov, D.L. (2013) Using a stable isotope to label seeds and seedlings of an invasive shrub, *Lonicera maackii*. *Invasive Plant Science and Management*, **6**, 112-117.
- Caut, S., Angulo, E. & Courchamp, F. (2008) Dietary shift of an invasive predator: rats, seabirds and sea turtles. *J Appl Ecol*, **45**, 428-437.
- Caut, S., Angulo, E. & Courchamp, F. (2009) Variation in discrimination factors of ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol*, **46**, 443-453.
- Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P. & Evershed, R.P. (2004) Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. *Oecologia*, **139**, 325-335.
- Cheesman, A.W. & Cernusak, L.A. (2016) Isoscapes: a new dimension in community ecology. *Tree Physiology*, **36**, 1456-1459.
- Cherel, Y., Fontaine, C., Jackson, G.D., Jackson, C.H. & Richard, P. (2009) Tissue, ontogenic and sex-related differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the oceanic squid *Todarodes filippovae* (Cephalopoda: Ommastrephidae). *Marine Biology*, **156**, 699-708.
- Cherel, Y. & Hobson, K.A. (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Marine Ecology Progress Series*, **329**, 281-287.
- Chevalier, R., Pelletier, G. & Gagnon, M. (1984) Sampling technique for collection of expired CO_2 in studies using naturally labeled ^{13}C in calves. *Canadian Journal of Animal Science*, **64**, 495-498.
- Chikaraishi, Y., Naraoka, H. & Poulson, S.R. (2004) Carbon and hydrogen isotopic fractionation during lipid biosynthesis in a higher plant (*Cryptomeria japonica*). *Phytochemistry*, **65**, 323-330.
- Chisholm, B.S., Nelson, D.E. & Schwarcz, H.P. (1982) Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. *Science*, **216**, 1131-1132.
- Church, M.R., Ebersole, J.L., Rensmeyer, K.M., Couture, R.B., Barrows, F.T. & Noakes, D.L.G. (2009) Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. *Canadian Journal of Fisheries and Aquatic Science*, **66**, 1-5.
- Cifuentes, L.A. & Salata, G.G. (2001) Significance of carbon isotope discrimination between bulk carbon and extracted phospholipid fatty acids in selected terrestrial and marine environments. *Organic Geochemistry*, **32**, 613-621.
- Comte, L., Cucherousset, J. & Olden, J.D. (2017) Global test of Eltonian niche conservatism of nonnative freshwater fish species between their native and introduced ranges. *Ecography*, **40**, 384-392.
- Coon, C.A.C., Brace, A.J., McWilliams, S.R., McCue, M.D. & Martin, L.B. (2014) Introduced and native congeners use different resource allocation strategies to maintain performance during infection. *Physiological and Biochemical Zoology*, **87**, 559-567.
- Coplen, T.B., Hopple, J.A., Bohlke, J.K., Peiser, H.S., Reider, S.E., Krouse, H.R., Rosman, K.J.R., Ding, T., Vocke, R.D., Revessz, K.M., Lamberty, A., Taylor, P. & De Bièvre, P. (2002) Compilation of minimum and maximum ratios of selected elements in naturally occurring terrestrial materials and reagents. (ed. USGS).
- Cucherousset, J. & Villeger, S. (2015) Quantifying the multiple facets of isotopic diversity: New metrics for stable isotope ecology. *Ecological Indicators*, **56**, 152-160.
- Curnutt, J.L. (2000) Host-area specific climatic-matching: similarity breeds exotics. *Biol Conservation*, **94**, 341-351.
- D'Antonio, C.M. & Vitousek, P.M. (1992) Biological invasions by exotic grasses, the grass fire cycle, and global change. *Ann Rev Ecol Syst*, **23**, 63-87.
- De Vink, J.-M., Slattery, S.M., Clark, R.G., Alisauskas, R.T. & Hobson, K.A. (2011) Combining stable isotope and body composition analyses to assess nutrient allocation strategies in breeding white-winged scoters. *Auk*, **128**.

- Deng, Y., Gui, Q., Dumont, M., Han, C., Deng, H., Yun, J. & Zhong, W. (2019) *Methylococcaceae* are the dominant active aerobic methanotrophs in a Chinese tidal marsh. *Environmental Science and Pollution Research*, **26**, 636-646.
- DeNiro, M.J. & Epstein, S. (1976) You are what you eat (plus a few per mil): the carbon isotope cycle in food chains. *Geological society of America Abstracts with Programs*, **8**, 834-835.
- Dibble, K.L., Pooler, P.S. & Meyerson, L.A. (2013) Impacts of plant invasions can be reversed through restoration: a regional meta-analysis of faunal communities. *Biological Invasions*, **15**, 1725-1737.
- Doronin, Y.K., Bednik, D.Y., Ivanov, A.A. & Kalistratova, E.N. (2012) Age-dependence of animal metabolism: an insight through the isotope fractionation prism. *Science Domain*.
- Doucett, R.R., Giberson, D.J. & Power, G. (1999) Parasitic association of *Nanocladius* (Diptera: Chironomidae) and *Pteronarcys biloba* (Plecoptera: Pteronarcyidae): insights from stable-isotope analysis. *Journal of North American Benthological Society*, **18**, 514-523.
- Durso, A.M. & French, S.S. (2017) Stable isotope tracers reveal a trade-off between reproduction and immunity in a reptile with competing needs. *Functional Ecology*, **10.1111/1365-2435.13002**, 1-9.
- Dzikiti, S., Schachtschneider, K., Naiken, V., Gush, M., Moses, G. & Le Maitre, D.C. (2013) Water relations and the effects of clearing invasive *Prosopis* trees on groundwater in an arid environment in the Northern Cape, South Africa. *J Arid Environments*, **90**, 103-113.
- Ens, E., Hutley, L.B., Rossiter-Ranchor, N.A., Douglas, M.M. & Setterfield, S.A. (2015) Resource-use efficiency explains grassy weed invasion in a low-resource savanna in north Australia. *Frontiers in Plant Science*, **6**, 50.
- Evans Ogden, L.J., Hobson, K.A. & Lank, D.B. (2004) Blood isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) turnover and diet-tissue fractionation factors in captive dunlin (*Calidris alpina pacifica*). *Auk*, **121**, 170-177.
- Evenepoel, P., Claus, D., Geypens, B., Hieli, M., Geboes, K., Rutgeerts, P. & Ghooos, Y. (1999) Amount and fate of egg protein escaping assimilation in the small intestine of humans. *American Journal of Physiology*, **277**, G935-G943.
- Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology*, **40**, 503-537.
- Fournier, A., Penone, C., Pennino, M.G. & Courchamp, F. (2019) Predicting future invaders and future invasions." Proceedings of the National Academy of Sciences. *PNAS*, **March 29**, 201803456.
- Fry, B. (2006) *Stable Isotope Ecology*. Springer, New York.
- Gannes, L.Z., Martinez del Rio, C. & Koch, P.L. (1998) Natural abundance variations in stable isotope isotopes and their potential use in animal physiological ecology. *Comparative Biochemistry and Physiology*, **119A**, 725-737.
- Gannes, L.Z., O'Brien, D.M. & Martinez del Rio, C. (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology*, **78**, 1271-1276.
- Garton, D.W., Payne, C.D. & Montoya, J.P. (2011) Flexible diet and trophic position of dreissenid mussels as inferred from stable isotopes of carbon and nitrogen. *Can J Fish Aquat Sci*, **62**, 1119-1129.
- Gaudichon, C., Mahe, S., Benamouzig, R., Luengo, C., Fouillet, H., Dare, S., Van Oyke, M., Ferriere, F., Rautureau, R. & Tome, D. (1999) Net postprandial utilization of [^{15}N]-labeled milk protein nitrogen is influenced by diet composition in humans. *Journal of Nutrition*, **129**, 890-895.
- Gay, L.-J., Schutz, Y., DiVetta, V., Schneiter, P., Tappy, L. & Jequier, E. (1994a) Measurement of $^{13}\text{CO}_2$ in expired air as an index of compliance to a high carbohydrate diet naturally enriched in ^{13}C . *International Journal of Obesity*, **18**, 591-595.
- Gay, L.J., Schneiter, P., Schutz, Y., Di Vetta, V., Jequier, E. & Tappy, L. (1994b) A non-invasive assessment of hepatic glycogen kinetics and post-absorptive gluconeogenesis in man. *Diabetologia*, **37**, 517-523.
- Geiser, F. (2004) Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology*, **66**, 239-274.
- Gratton, C. & Forbes, A.E. (2006) Changes in $\delta^{13}\text{C}$ stable isotopes in multiple tissues of insect predators fed isotopically distinct prey. *Oecologia*, **147**, 615-624.

- Graves, G.R., Romanek, C.S. & Rodriguez-Navarro, A. (2002) Stable isotope signature of philopatry and dispersal in a migratory songbird. *PNAS*, **99**, 8096-8100.
- Grey, J. & Jackson, M.C. (2012) 'Leaves and eats shoots': direct terrestrial feeding can supplement invasive red swamp crayfish in times of need. *PlosOne*, **7**, e42575.
- Grey, J., Waldron, S. & Hutchinson, R. (2004) The utility of carbon and nitrogen isotope analyses to trace contributions from fish farms to the receiving communities of freshwater lakes: a pilot study in Esthwaite Water, UK. *Hydrobiologia*, **524**, 253-262.
- Guisan, A., Petitpierre, B., Broennimann, O., Daehler, C. & Kuasser, C. (2014) Unifying niche shift studies: insights from biological invasions. *Trends Ecol Evol*, **29**, 260-268.
- Haichar, F.E., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T. & Achouak, W. (2008) Plant host habitat and root exudates shape soil bacterial community structure. *ISME Journal*, **2**, 1221-1230.
- Hammerschlag-Peyer, C.M., Yeager, L.A., Araujo, M.S. & Layman, C.A. (2011) A hypothesis-testing framework for studies investigating ontogenetic niche shifts using stable isotopes. *PlosOne*, **6**, e27104.
- Hawkins, A.J.S. (1985) Relationships between the synthesis and breakdown of protein, dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus edulis* L. *Oecologia*, **66**, 42-49.
- Hayes, J.M. (2001) Fractionation of carbon and hydrogen isotopes in biosynthetic processes. *Reviews in Mineralogy and Geochemistry*, **43**, 225-277.
- Heinrich, K. & Collins, L. (2016) Determining the geographical origin of Asian longhorn beetle (*Anoplophora glabripennis*) specimens using stable isotope and trace element analyses. *Pest Management Science*, **73**, 967-975.
- Hellmann, C., Werner, C. & Oldeland, J. (2016) A Spatially Explicit Dual-Isotope Approach to Map Regions of Plant-Plant Interaction after Exotic Plant Invasion. *PlosOne*, **11**, e0159403.
- Hershey, A.E. & Pastor, J. (1993) Stable isotopes resolve the drift paradox for *Baetis* mayflies in an arctic river. *Ecology*, **74**, 2315-2325.
- Hette-Tronquart, N. (2019) Isotopic niche is not equal to trophic niche. *Ecology Letters*.
- Higgins, S.I. & Richardson, D.M. (1999) Predicting plant migration rates in a changing world: the role of long-distance dispersal. *American Naturalist*, **153**, 464-475.
- Hill, J.M., Jones, R.W., Hill, M.P. & Weyl, O.L.F. (2015) Comparisons of isotopic niche widths of some invasive and indigenous fauna in a South African river. *Freshwater Biology*, **60**, 893-902.
- Hobson, K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314-326.
- Hobson, K.A., Schell, D.M., Renouf, D. & Noseworthy, E. (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Canadian Journal of Fisheries and Aquatic Science*, **53**, 528-533.
- Hobson, K.A. & Wassenaar, L.I. (1999) Stable isotope ecology: an introduction. *Oecologia*, **120**, 312-313.
- Hohtola, E. (2012) Thermoregulatory adaptations to starvation in birds. *Comparative Physiology of Fasting, Starvation, and Food Limitation* (ed. M.D. McCue), pp. 155-170. Springer-Verlag, New York.
- Holmes, P.M., Richardson, D.M., Esler, K.J., Witkowski, E.T.F. & Fourie, S. (2005) A decision-making framework for restoring riparian zones degraded by invasive alien plants in South Africa. *South African Journal of Science*, **101**.
- Holmstrup, M., Hedlund, K. & Boriss, H. (2002) Drought acclimation and lipid composition in *Folsomia candida*: implications for cold shock, heat shock and acute desiccation stress. *Journal of Insect Physiology*, **48**, 961-970.
- Hood-Nowotny, R. & Knols, B.G. (2007) Stable isotope methods in biological and ecological studies of arthropods. *Entomologia Experimentalis et Applicata*, **124**, 3-16.

- Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., Schlaeppli, K. & Erb, M. (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat Commun*, **9**, 2738.
- Hufbauer, R.A., Facon, B., Ravigne, V., Turgeon, J., Foucaud, J., Lee, C.E., Rey, O. & Estoup, A. (2012) Anthropogenically induced adaptation to invade (AIAI): Contemporary adaptation to human-altered habitats within the native range can promote invasions. *Evol Appl*, **5**, 89-101.
- Hui, C. & Richardson, D.M. (2017) *Invasion dynamics*. Oxford University Press, Oxford.
- Hungate, B.A., Kearns, D.N., Ogle, K., Caron, M., Marks, J.C. & Rogg, H.W. (2016) Hydrogen isotopes as a sentinel of biological invasion by the Japanese beetle, *Popilla japonica* (Newman). *PlosOne*, **11**, e0149599.
- IAEA (2000) Abundance and fractionation of stable isotopes. *Environmental Isotopes in the Hydrological Cycle* (ed. W.H. Mook), pp. 31-48. International Atomic Energy Agency.
- Inger, R. & Bearhop, S. (2008) Applications of stable isotope analyses to avian ecology. *Ibis*, **150**, 447-461.
- Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011) Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *J Anim Ecol*, **80**, 595-602.
- Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J. (2012) Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PlosOne*, **7**, e31757.
- Jackson, M.C., Evangelista, C., Zhao, T., Lecerf, A., Britton, J.R. & Cucherousset, J. (2017a) Between-lake variation in the trophic ecology of an invasive crayfish. *Freshwater Biology*, **62**, 1501-1510.
- Jackson, M.C., Grey, J.M., K., Britton, J.R. & Donohue, I. (2016a) Dietary niche construction when invaders meet natives: evidence from freshwater decapods. *J Anim Ecol*, **85**, 1098-1107.
- Jackson, M.C., Wasserman, R.J., Grey, J., Ricciardi, A., Dick, J.T.A. & Alexander, M.E. (2017b) Novel and disrupted trophic links following invasion in freshwater ecosystems. *Advances in Ecol Res*, **57**, 55-97.
- Jackson, M.C., Woodford, D.J., Bellingan, T.A., Weyl, O.L.F., Potgieter, M.J., Rivers-Moore, N.A., Ellender, B.R., Fourie, H.E. & Chimimba, C.T. (2016b) Trophic overlap between fish and riparian spiders: potential impacts of an invasive fish on terrestrial consumers. *Ecology and Evolution*, **6**, 1745-1752.
- Jeschke, J.M., Bacher, S., Blackburn, T.M., Dick, J.T.A., Essl, F., Evans, T., Gaertner, M., Hulme, P.E., Kuhn, I., A., M., Pergl, J., Pysek, P., Rabitsch, W., Ricciardi, A., Richardson, D.M., Sendek, A., Vila, M., Winter, M. & S., K. (2014) Defining the impact of non-native species. *Conservation Biology*, **28**, 1188-1194.
- Jeschke, J.M. & Heger, T. (2018) *Invasion biology. Hypotheses and evidence*. CABI, Boston.
- Kamenova, S., Bartley, T.J., Bohan, D.A., Boutain, J.R., Colautti, R.I., Domaizon, I., Fontaine, C., Lemainque, A., Le Viol, I., Mollot, G. & Perga, M.E. (2017) Invasions toolkit: Current methods for tracking the spread and impact of invasive species. *Advances in Ecol Res*, pp. 85-182. Academic Press.
- Kamutando, C.N., Vikram, S., Kamgan-Nkuekam, G., Makhallanyane, T.P., Greve, M., Le Roux, J.J., Richardson, D.M., Cowan, D. & Valverde, A. (2017) Soil nutritional status and biogeography influence rhizosphere microbial communities associated with the invasive tree *Acacia dealbata*. *Scientific Reports*, **7**, 6472.
- Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends Ecol Evol*, **17**, 164-170.
- Keet, J.H., Ellis, A.G., Hui, C. & Le Roux, J.J. (2017) Legume-rhizobium symbiotic promiscuity does not determine plant invasiveness. *Annals of Botany*, **119**.
- Kelly, J.F. (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, **78**, 1-27.

- 999 Kenis, M., Auger-Rozenberg, M.-A., Roques, A., Timms, L., Pere, C., Cock, M.J.W., Settele, J.,
1000 Augustin, S. & Lopez-Vaamonde, C. (2009) Ecological effects of invasive alien insects. *Biol*
1001 *Invasions*, **11**, 21-45.
- 1002 Khalilieh, A., McCue, M.D. & Pinshow, B. (2015) House sparrows have two phases of fuel use during
1003 fasting, rather than the classic three. *Integrative and Comparative Biology*, **55**, E283.
- 1004 Kindscher, K. & Tieszen, L.L. (1998) Floristic and soil organic matter changes after five and thirty-five
1005 years of native tallgrass prairie restoration. *Restoration Ecology*, **6**, 181-196.
- 1006 Kiszka, J., Oremus, M., Richard, P., Poole, M. & Ridoux, V. (2010) The use of stable isotope analyses
1007 from skin biopsy samples to assess trophic relationships of sympatric delphinids off Moorea
1008 (French Polynesia). *J Exp Mar Biol Ecol*, **395**, 48-54.
- 1009 Kumschick, S., Bacher, S., Evans, T., Markova, Z., Pergl, J., Pysek, P., Vaes-Petignat, S., vander Veer,
1010 G., Vila, M. & Nentwig, W. (2015) Comparing impacts of alien plants and animals in Europe
1011 using a standard scoring system. *J Appl Ecol*, **52**, 552-561.
- 1012 Lambrecht-McDowell, S.C. & Radosevich, S.R. (2005) Population demographics and trade-offs to
1013 reproduction of an invasive and noninvasive species of *Rubus*. *Biol Invasions*, **7**, 281-295.
- 1014 Layman, C.A., Arrington, D.A., Montana, C.G. & Post, D.M. (2007) Can stable isotope ratios provide for
1015 community-wide measures of trophic structure? *Ecology*, **88**, 42-48.
- 1016 Le Roux, J.J., Hui, C., Keet, J.H. & Ellis, A.G. (2017) Co-introduction vs ecological fitting as pathways
1017 to the establishment of effective mutualisms during biological invasions. *New Phytologist*, **215**,
1018 1354-1360.
- 1019 Levin, E., McCue, M.D. & Davidowitz, G. (2017) More than just sugar: allocation of nectar amino acids
1020 and fatty acids in a Lepidopteran. *Proc Roy Soc London B*, **284**.
- 1021 Levine, J.M., Pachepsky, E., Kendall, B.E., Yelenik, S.G. & Lambers, J.H.R. (2006) Plant–soil feedbacks
1022 and invasive spread. *Ecology Letters*, **9**, 1005-1014.
- 1023 Mack, M.C., D'Antonio, C.M. & Ley, R.E. (2001) Alteration of ecosystem nitrogen dynamics by exotic
1024 plants: a case study of C4 grasses in Hawaii. *Ecological Applications*, **11**, 1323-1335.
- 1025 MacNeale, K.H., Peckarsky, B.L. & Likens, G.E. (2004) Contradictory results from different methods for
1026 measuring direction of insect flight. *Freshwater Biology*, **49**, 1260-1268.
- 1027 MacNeale, K.H., Peckarsky, B.L. & Likens, G.E. (2005) Stable isotopes identify dispersal patterns of
1028 stonefly populations living along stream corridors. *Freshwater Biology*, **50**, 1117-1130.
- 1029 Magadlela, A., Beukes, C., Venter, F., E., S. & Valentine, A.J. (2017) Does P deficiency affect nodule
1030 bacterial composition and N source utilisation in a legume from nutrient-poor Mediterranean-type
1031 ecosystems? . *Soil Biology and Biochemistry*, **104**, 164-174.
- 1032 Magadlela, A., Perez-Fernandez, M., Kleinert, A., Dreyer, L.L. & Valentine, A.J. (2016) Source of
1033 inorganic N affects the cost of growth in a legume tree species (*Virgilia divaricata*) from the
1034 Mediterrean-type Fynbos ecosystem. *J Plant Ecol*, **9**, 752-761.
- 1035 Magozzi, S., Yool, A., Vander Zanden, H.B., Wunder, M.B. & Trueman, C.N. (2017) Using ocean
1036 models to predict spatial and temporal variation in marine carbon isotopes. *Ecosphere*, **8**, e01763.
- 1037 Maguas, C., Rascher, K.G., Martins-Loucao, A., Carvalho, P., Pinho, P., Ramos, M., Correia, O. &
1038 Werner, C. (2011) Responses of woody species to spatial and temporal ground water changes in
1039 coastal sand dune systems. *Biogeosciences*, **8**, 3823-3832.
- 1040 Maguire, C.A. & Grey, J. (2006) Determination of zooplankton dietary shift following a zebra mussel
1041 invasion, as indicated by a stable isotope analysis. *Freshwater Biology*, **51**, 1310-1319.
- 1042 Maron, J.L. & Vila, M. (2001) When do herbivores affect plant invasion? Evidence for the natural
1043 enemies and biotic resistance hypotheses. *Oikos*, **93**, 361-373.
- 1044 Martinez del Rio, C., Wolf, N., Carleton, S.A. & Gannes, L.Z. (2009) Isotopic ecology ten years after a
1045 call for more laboratory experiments. *Biological Reviews*, **84**, 91-111.
- 1046 Mason, R.A., Cooke, J., Moles, A.T. & Leishman, M.R. (2008) Reproductive output of invasive versus
1047 native plants. *Global Ecology and Biogeography*, **17**, 633-640.
- 1048 McAllen, B.M. & Geiser, F. (2014) Torpor during reproduction in mammals and birds: dealing with an
1049 energetic conundrum. *Integrative and Comparative Biology*, **54**, 516-532.

- McCarthy, M.D., Benner, R., Lee, C. & Fogel, M.L. (2007) Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochimica et Cosmochimica Acta*, **71**, 4727-4744.
- McClelland, J.W. & Montoya, J.P. (2002) Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology*, **83**, 2173-2180.
- McCue, M.D. (2008) Fatty acid analyses may provide insight into the progression of starvation among squamate reptiles. *Comparative Biochemistry and Physiology*, **151A**, 239-246.
- McCue, M.D. (2010) Starvation physiology: reviewing the different strategies animals use to survive a common challenge *Comparative Biochemistry and Physiology*, **156A**, 1-18.
- McCue, M.D. (2011) Tracking the oxidative and non-oxidative fates of isotopically labeled nutrients in animals. *BioScience*, **61**, 217-230.
- McCue, M.D. (2013) Direct measurement of starvation-induced shifts in endogenous fuel oxidation in mice. *Integrative and Comparative Biology*, **53**, E141.
- McCue, M.D., Amaya, J.A., Yang, A.S., Erhardt, E.B., Wolf, B.O. & Hanson, D.T. (2013a) Targeted ^{13}C enrichment of lipid and protein pools in the body reveals circadian changes in oxidative fuel mixture during prolonged fasting: a case study using Japanese quail. *Comp Biochem Physiol*, **166A**, 546-554.
- McCue, M.D., Arquisola, B., Albach, E. & Pollock, E.D. (2013b) Hens produce artificially enriched ^{13}C egg proteins for metabolic tracer studies. *International Journal of Biology*, **5**, 69-84.
- McCue, M.D., Guzman, R.M., Passemant, C.A. & Davidowitz, G. (2015) How do insects rely on endogenous protein and lipid resources during lethal bouts of starvation? A new application for ^{13}C -breath testing. *PlosOne*, **10**, e0140053.
- McCue, M.D., Passemant, C.A. & Guzman, R.M. (2015) Digesting pythons oxidize the proteins in their meals and save the lipids for later. *Journal of Experimental Biology*, **215**, 2089-2096.
- McCue, M.D., Sivan, O., McWilliams, S.R. & Pinshow, B. (2010) Tracking the oxidative kinetics of carbohydrates, amino acids, and fatty acids in the house sparrow using exhaled $^{13}\text{CO}_2$. *Journal of Experimental Biology*, **213**, 782-789.
- McCue, M.D., Smith, A., McKinney, R., Rewald, B., Pinshow, B. & McWilliams, S.R. (2011) A mass balance approach to identify and compare differential routing of ^{13}C -labeled carbohydrates, lipids, and proteins in vivo. *Physiological and Biochemical Zoology*, **84**, 506-513.
- McCue, M.D. & Welch Jr, K.C. (2016) ^{13}C -Breath testing in animals: Theory, applications, and future directions. *J Comp Physiol*, **186B**, 265-285.
- McKechie, A.E. (2004) Stable isotopes: powerful new tools for animal ecologists. *South African J Sci*, **100**, 131-134.
- McKechie, A.E. & Mzilikazi, N. (2011) Heterothermy in Afrotropical mammals and birds: a review. *Integrative and Comparative Biology*, **51**, 349-363.
- Metges, C., Kempe, K. & Schmidt, H.-L. (1990) Dependence of the carbon-isotope contents of breath carbon dioxide, milk, serum and rumen fermentation products on the $\delta^{13}\text{C}$ value of food in dairy cows. *British J Nutr*, **63**, 187-196.
- Morris, L.L., Walck, J.L. & Hidayati, S.N. (2002) Growth and reproduction of the invasive *Ligustrum sinense* and native *Forestiera ligustrina* (Oleaceae): implications for the invasion and persistence of a nonnative shrub. *163*, **6**, 1001-1010.
- Mothapo, N.P. & Wossler, T.C. (2017) Patterns of floral resources use by two dominant ant species in a biodiversity hotspot. *Biol Invasions*, **19**, 955-969.
- Muller, C., Assimacopoulos-Jeannet, F., Mosimann, F., Schneiter, P., Riou, J.P., Pachiardi, C., Felber, J.P., Jequier, E., Jeanrenaud, B. & Tappy, L. (1997) Endogenous glucose production, gluconeogenesis and liver glycogen concentration in obese non-diabetic patients. *Diabetologia*, **40**, 463-468.
- Munoz-Garcia, A., Aamidor, S., McCue, M.D., McWilliams, S.R. & Pinshow, B. (2012) Allocation of endogenous and dietary protein in the reconstitution of the gastrointestinal tract in migratory blackcaps at stopover sites. *Journal of Experimental Biology*, **215**, 1069-1075.

- 1101 Murphy, B.P., Bowman, D.M.J.S. & Gagan, M.K. (2007) The interactive effect of temperature and
1102 humidity on the oxygen isotope composition of kangaroos. *Functional Ecology*, **21**, 757-766.
- 1103 Murray, I. & Wolf, B.O. (2012) Tissue carbon incorporation rates and diet-to-tissue discrimination in
1104 ectotherms: tortoises are really slow. *Physiol Biochem Zool*, **85**, 96-105.
- 1105 Murray, I. & Wolf, B.O. (2013) Desert tortoise (*Gopherus agassizii*) dietary specialization decreases
1106 across a precipitation gradient. *PlosOne*, **8**, e66505.
- 1107 Mustonen, A.-M., Bowman, J., Sadowski, C., Nituch, L.A., Bruce, L., Halonen, T., Puukka, K.,
1108 Rouvinen-Watt, K., Aho, J. & Nieminen, P. (2013) Physiological adaptations to prolonged fasting
1109 in the overwintering striped skunk (*Mephitis mephitis*). *Comp Biochem Physiol*, **166A**, 555-563.
- 1110 Mutirwara, R., Radloff, F.G.T. & Codron, D. (2017) Growth rate and stable carbon and nitrogen isotopic
1111 tropic discrimination factors of lion and leopard whiskers. *Rapid Comm Mass Spectrom*, **32**, 33-
1112 47.
- 1113 Nardoto, G.B., Silva, S., Kendall, C., Ehleringer, J.R., Chesson, L.A., Ferraz, E.S.B., Moreira, M.Z.,
1114 Ometto, J.P.H.B. & Martinelli, L.A. (2006) Geographical patterns of human diet derived from
1115 stable-isotope analysis of fingernails. *American Journal of Physical Anthropology*, **131**, 137-146.
- 1116 Nienstedt, K.M. & Poehling, H.-M. (2000) ¹⁵N-marked aphids for predation studies under field
1117 conditions. *Entomologica Experimentalis et Applicata*, **94**, 319-323.
- 1118 Nienstedt, K.M. & Poehling, H.-M. (2004) Invertebrate predation of ¹⁵N-marked prey in semi-field wheat
1119 enclosures. *Entomologica Experimentalis et Applicata*, **112**, 191-200.
- 1120 Nyssen, F., Brey, T., Dauby, P. & Graeve, M. (2005) Trophic position of Antarctic amphipods - enhanced
1121 analysis by a 2-dimensional biomarker assay. *Marine Ecology Progress Series*, **300**, 135-145.
- 1122 O'Brien, D.M. (2015) Stable isotope ratios as biomarkers of diet for health reserach. *Ann Rev Nutr*, **35**,
1123 565-594.
- 1124 O'Brien, D.M., Fogel, M.L. & Boggs, C.L. (2002) Renewable and nonrenewable resources: amino acid
1125 turnover and allocation to reproduction in lepidoptera. *PNAS*, **99**, 4413-4418.
- 1126 O'Brien, D.M., Schrag, D.P. & Martinez del Rio, C. (2000) Allocation to reproduction in a hawkmoth: a
1127 quantitative analysis using stable carbon isotopes. *Ecology*, **81**, 2822-2831.
- 1128 O'Leary, M.H. (1988) Carbon isotopes in photosynthesis. *BioScience*, **38**, 328-336.
- 1129 O'Leary, M.H., Madhavan, S. & Paneth, P. (1992) Physical and chemical basis of carbon isotope
1130 fractionation in plants. *Plant, Cell and Environment*, **15**, 1099-1104.
- 1131 Olsson, K., Stenroth, P., Nystrom, P. & Graneli, W. (2009) Invasions and niche width: does niche width
1132 of an introduced crayfish differ from a native crayfish? *Freshwater Biology*, **54**, 1731-1740.
- 1133 Ouborg, N.J., Piquot, Y. & van Groenendael, J.M. (1999) Population genetics, molecular markers, and the
1134 study of dispersal in plants. *Journal of Ecology*, **87**, 551-569.
- 1135 Parker, I.M., Simberloff, D., Lonsdale, W., Goodell, K., Wonham, M., Kareiva, P., Williamson, M., Von
1136 Holle, B., Moyle, P., Byers, J. & Goldwasser, L. (1999) Impact: toward a framework for
1137 understanding the ecological effects of invaders. *Biol Invasions*, **1**, 3-19.
- 1138 Paynter, Q., Csurhes, S.M., Heard, T.A., Ireson, J., Julien, M.H., Lloyd, J., Lonsdale, W.M., Palmer,
1139 W.A., Sheppard, A.W. & van Klinken, R.D. (2003) Worth the risk? Introduction of legumes can
1140 cause more harm than good: an Australian perspective. *Austrian Sytematic Botany*, **16**, 81-88.
- 1141 Pekarsky, S., Angert, A., Haese, B., Werner, M., Hobson, K.A. & Nathan, R. (2015) Enriching the
1142 isotopic toolbox for migratory connectivity analysis: a new approach for migratory species
1143 breeding in remote or unexplored areas. *Diversity and Distribution*, 1-12.
- 1144 Peperkorn, R., Werner, C. & Beyschlag, W. (2005) Phenotypic plasticity of an invasive *Acacia* versus
1145 two native Mediterranean species. *Functional Plant Biology*, **32**, 933-944.
- 1146 Peterson, B.J. & Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and*
1147 *Systematics*, **18**, 293-320.
- 1148 Phillips, D.L. (2012) Converting isotope values to diet composition: the use of mixing models. *Journal of*
1149 *Mammalogy*, **93**, 342-352.
- 1150 Phillips, D.L. & Gregg, J.W. (2003) Source partitioning using stable isotopes: coping with too many
1151 sources. *Oecologia*, **136**, 261-269.

- Plasman, M., McCue, M.D., Reynoso, V.H., Terblanche, J.S. & Clusella-Trullas, S. (2019) Environmental temperature alters the overall digestive energetics and differentially affects dietary protein and lipid use in a lizard. *J Exp Biol*, **222**, jeb194480.
- Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703-718.
- Potapov, A.M., Tiunov, A.V. & Scheu, S. (2018) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. *Biological Reviews*, in press.
- Prochazka, P., Van Wilgenburg, S.L., Neto, J.M., Yosef, R. & Hobson, K.A. (2013) Using stable hydrogen isotopes ($\delta^2\text{H}$) and ring recoveries to trace natal origins in a Eurasian passerine with a migratory divide. *Journal of Avian Biology*, **44**, 541-550.
- Quillfeldt, P., Bugoni, L., McGill, R.A., Masello, J.F. & Furness, R.W. (2008) Differences in stable isotopes in blood and feathers of seabirds are consistent across species, age and latitude: implications for food web studies. *Marine Biology*, **155**, 593-598.
- Radajewski, S., Ineson, P., Parekh, N.R. & Murrell, J.C. (2000) Stable-isotope probing as a tool in microbial ecology. *Nature*, **403**, 646-649.
- Ranius, T. (2006) Measuring the dispersal of saproxylic insects: A key characteristic for their conservation. *Population Ecology*, **48**, 177-188.
- Rascher, K.G., Grobe-Stoltenberg, A., Maguas, C., Meira-Neto, J.A.A. & Werner, C. (2011) *Acacia longifolia* invasion impacts vegetation structure and regeneration dynamics in open dunes and pine forests. *Biological Invasions*.
- Rascher, K.G., Hellmann, C., Maguas, C. & Werner, C. (2012) Community scale ^{15}N isoscapes: tracing the spatial impact of an exotic N_2 -fixing invader. *Ecology Letters*, **15**, 484-491.
- Reich, K.J., Bjorndal, K.A. & Martinez Del Rio, C. (2008) Effects of growth and tissue type on the kinetics of ^{13}C and ^{15}N incorporation in a rapidly growing ectotherm. *Oecologia*, **155**, 6510663.
- Ricciardi, A., Blackburn, T.M., Carlton, J.T., Dick, J.T., Hulme, P.E., Iacarella, J.C., Jeschke, J.M., Liebhold, A.M., Lockwood, J.L., MacIsaac, H.J. & Pysek, P. (2017) Invasion science: a horizon scan of emerging challenges and opportunities. *Trends Ecol Evol*, **32**, 464-474.
- Richards, R.A. (1996) Defining selection criteria to improve yield under drought. *Plant Growth Regul*, **20**.
- Richardson, D.M. (2011) *Fifty years of invasion ecology. The legacy of Charles Elton*. Wiley-Blackwell, Oxford.
- Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J. & Rejmanek, M. (2000) Plant invasions: the role of mutualisms. *Biol Reviews*, **75**, 65-93.
- Richardson, D.M. & Pysek, P. (2006) Plant invasions – merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography*, **30**, 409-431.
- Roques, A. (2010) Alien forest insects in a warmer world and a globalised economy: Impacts of changes in trade, tourism, and climate on forest biosecurity. *New Zeal J Sci*, **40**, 77-94.
- Roques, A., Auger-Rozenberg, M.-A., Blackburn, T.M., Garnas, J., Pysek, P., Rabitsch, W., Richardson, D.M., Wingfield, M.J., Liebhold, A.M. & Duncan, R.P. (2016) Temporal and interspecific variation in rates of spread for insect species invading Europe during the last 200 years. *Biol Invasions*, **18**, 907-920.
- Rosenblatt, A.E. & Heithaus, M.R. (2013) Slow isotope turnover rates and low discrimination values in the American alligator: implications for interpretation of ectotherm stable isotope data. *Physiol Biochem Zool*, **86**, 137-148.
- Rubenstein, D.R. & Hobson, K.A. (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology and Evolution*, **19**, 256-263.
- Ruf, T. & Geiser, F. (2014) Daily torpor and hibernation in birds and mammals. *Biological Reviews*, **90**, 891-926.
- Sagouis, A., Cucherousset, J., Villeger, S., Santoul, F. & Boulentreau, S. (2015) Non-native species modify the isotopic structure of freshwater fish communities across the globe. *Ecography*, **38**, 979-985.

- Saito, T., Tsukumo, M., Elbasit, A.M.A., Yasuda, H., Kawai, T., Matsuo, M., Inosako, K., Archara, K., Bibiker, A.E., Hamd, A.A. & Nawata, H. (2014) Estimation of water sources of invasive tree species in arid environments by oxygen stable isotope analysis. *J Arid Land Studies*, **24**, 29-32.
- Schell, D.M., Saupe, S.M. & Haubenstock, N. (1989) Bowhead whale (*Balaena mysticus*) growth and feeding as estimated by $\delta^{13}\text{C}$ techniques. *Marine Biology*, **103**, 433-443.
- Schimmelmann, A. (2011) Carbon, nitrogen and oxygen stable isotope ratios in chitin. *Chitin. Topics in Geobiology* (ed. N. Gupta), pp. 81-103. Springer, Dordrecht.
- Schmidt, S.N., Olden, J.D., Solomon, C.T. & Vander Zanden, M.J. (2007) Quantitative approaches to the analysis of stable isotope food web data. *Ecology*, **88**, 2793-2802.
- Seebens, H., Blackburn, T.M., Dyer, E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pysek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jager, H., Kartesz, J., Kenis, M., Kreft, H., Kuhn, I., Lenzer, B., Liebhold, A.M., Mosena, A., Moser, D., Roy, H.E., Scalera, R., Schindler, S., Stajero, K., Tokarska-Guzik, B., van Kleunen, M., Walker, K., Weigelt, P., Yamanka, T. & Essl, F. (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications*, **8**, 14435.
- Seibt, U., Rajabi, A., Griffiths, H. & Berry, J.A. (2008) Carbon isotopes and water use efficiency: sense and sensitivity *Oecologia*, **155**, 441-454.
- Simberloff, D. (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters*, **9**, 912-919.
- Simberloff, D. & Von Holle, B. (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biol Invasions*, **1**, 21-32.
- Sponheimer, M., Robinson, T.F., Roeder, B.L., Passey, B.H., Ayliffe, L.K., Cerling, T.E., Dearing, M.D. & Ehleringer, J.R. (2003) An experimental study of nitrogen flux in llamas: is ^{14}N preferentially excreted? *Journal of Archaeological Science*, **30**, 1649-1655.
- Storey, K.B. & Storey, J.M. (1990) Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Quarterly Review of Biology*, **65**, 145-174.
- Stratton, L.C. & Goldstein, G. (2001) Carbon uptake, growth and resources-use efficiency in one invasive and six native Hawaiian dry forest tree species. *Tree Physiology*, **21**, 1327-1334.
- Strayer, D.L., Eviner, V.T., Jeschke, J.M. & Pace, M.L. (2006) Understanding the long-term effects of species invasions *Trends Ecol Evol*, **21**, 645-651.
- Tabak, M.A., Anderson, O.R.J., Robb, G., Poncet, S., Passfield, K., Martinez, M.G. & Martinez Del Rio, C. (2016) Comparative isotopic natural history of two native passerines (*Troglodytes cobbi* and *Cinclodes antarcticus*) and the invasive rats (*Rattus norvegicus*) that extirpate them. *Austral Ecology*, **41**, 622-632.
- Tanis, A.A., Rietveld, T., Wattimena, J.L.D., van den Berg, J.W.O. & Swart, G.R. (2003) The $^{13}\text{CO}_2$ breath test for liver glycogen oxidation after 3-day labeling of the liver with a naturally ^{13}C -enriched diet. *Nutrition*, **19**, 432-437.
- Tanis, A.A., van den Berg, J.W.O., Kroneman, B.R., Wattimena, J.L.D., Rietveld, T., Nieland, B.H.B. & Swart, G.R. (1998) Human liver glycogen metabolism assessed with a ^{13}C -enriched diet and a $^{13}\text{CO}_2$ breath test. *Euro J Clin Invest*, **28**, 466-474.
- Terzer, S., Wassenaar, L.I., Araguas-Arags, L.J. & Aggarwal, P.K. (2013) Global isoscapes for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in precipitation: improved prediction using regionalized climatic regression models. *Hydrology and Earth System Sciences*, **17**, 4713-4728.
- Thompson, D.R., Bury, S.J., Hobson, K.A., Wassenaar, L.I. & Shannon, J.P. (2005) Stable isotopes in ecological studies. *Oecologia*, **144**, 517-519.
- Thuiller, W., Richardson, D., Rouget, M., Proches, S. & Wilson, J. (2006) Interactions between environment, species traits, and human uses describe patterns of plant invasions. *Ecology*, **87**, 1755-1769.

- 1253 Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. & Slade, N.A. (1983) Fractionation and turnover of stable
1254 carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia*, **57**, 32-37.
- 1255 Tillberg, C.V., Holoway, D.A., LeBrun, E.G. & Suarez, A.V. (2007) Trophic ecology of invasive
1256 Argentine ants and their native and introduced ranges. *PNAS*, **104**, 20856-20861.
- 1257 Trakhtenbrot, A., Nathan, R., Perry, G. & Richardson, D.M. (2005) The importance of long-distance
1258 dispersal in biodiversity conservation. *Diversity and Distributions*, **11**, 173-181.
- 1259 Turner, T.F., Collyer, M.L. & Krabbenhoft, T.J. (2010) A general hypothesis-testing framework for stable
1260 isotope ratios in ecological studies. *Ecology*, **91**, 2227-2233.
- 1261 Ulm, F., Hellmann, C., Cruz, C. & Maguas, C. (2016) N/P imbalance as a key driver for the invasion of
1262 oligotrophic dune systems by a woody legume. *Oikos*, **126**, 231-240.
- 1263 Ulm, F., Jacinto, J., Cruz, C. & Maguas, C. (2017) How to outgrow your native neighbor? Belowground
1264 changes under native shrubs at an early stage of invasion. *Land Degredation & Development*, **28**,
1265 2380-2388.
- 1266 Unkovich, M. (2013) Isotope discrimination provides new insight into biological nitrogen fixation. *New*
1267 *Phytologist*, **198**, 643-646.
- 1268 Van Dyke, J.U. & Beaupre, S.J. (2012) Stable isotope tracer reveals that viviparous snakes transport
1269 amino acids to offspring during gestation. *J Exp Biol*, **215**, 760-765.
- 1270 Van Dyke, J.U., Beaupre, S.J. & Kreider, D.L. (2012) Snakes allocate amino acids acquired during
1271 vitellogenesis to offspring: are capital and income breeding consequences of variable foraging
1272 success? *Biol J Linn Soc*, **106**, 390-404.
- 1273 Vander Zanden, H.B., Soto, D.X., Bowen, G.J. & Hobson, K.A. (2016) Expanding the isotopic toolbox:
1274 applications of hydrogen and oxygen stable isotope ratios to food web studies. *Frontiers in*
1275 *Ecology and Evolution*, **4**, 1-19.
- 1276 Vander Zanden, M.J., Casselman, J.M. & Rasmussen, J.B. (1999) Stable isotope evidence for the food
1277 web consequences of species in lakes. *Nature*, **401**, 464-467.
- 1278 Vander Zanden, M.J., Clayton, M.K., Moody, E.K., Solomon, C.T. & Weidel, B.C. (2015) Stable isotope
1279 turnover and half-life in animal tissues: a literature synthesis. *PlosOne*.
- 1280 Vas, A.S., Kueffer, C., Kull, C.A., Richardson, D.M., Schindler, S., Munoz-Pajeres, A.J., Vicente, J.R.,
1281 Martins, J., Hui, C., Kuhn, I. & Honrado, J.P. (2017) The progress of interdisciplinarity in
1282 invasion science. *Ambio*, **46**, 428-442.
- 1283 Vinagre, C., Silva, R., Mendonca, V., Flores, A.A., Baeta, A. & Marques, J.C. (2018) Food web
1284 organization following the invasion of habitat-modifying *Tubastraea* spp. corals appears to
1285 favour the invasive borer bivalve *Leiosolenus aristatus*. *Ecological Indicators*, **85**, 1204-1209.
- 1286 Voigt, C.C. (2009) Studying animal diets *in situ* using portable stable isotope analyzers. *Biotropica*, **41**,
1287 271-274.
- 1288 Voigt, C.C., Thalwitzer, S., Melzheimer, J., Blanc, A.-S., Jago, M. & Wachter, B. (2014) The conflict
1289 between cheetahs and humans on Namibian farmland elucidated by stable isotope diet analyses.
1290 *PlosOne*, **8**, e101917.
- 1291 Waring, B.G., Alvarez-Cansino, L., Barry, K.E., Becklund, K.K., Dale, S., Gei, M.G., Keller, A.B.,
1292 Lopez, O.R., Markesteijn, L., Mangan, S., Riggs, C.E., Rodriguez-Ronderos, M.E., Segnitz,
1293 R.M., Schnitzer, S.A. & Powers, J.S. (2015) Pervasive and strong effects of plants on soil
1294 chemistry: a meta-analysis of individual plant 'Zinke' effects. *Proc Biol Sci*, **282**, 1812.
- 1295 Warner, D.A., Bonnet, X., Hobson, K.A. & Shine, R. (2008) Lizards combine stored energy and recently
1296 acquired nutrients flexibly to fuel reproduction. *J Anim Ecol*, **77**, 1242-1249.
- 1297 Welch Jr, K.C., Perronet, F., Voigt, C.C., Hatch, K. & McCue, M.D. (2016) Combining respirometry with
1298 stable isotopes to investigate fuel use in animals. *Annals of the New York Academy of Sciences*,
1299 **1365**, 15-32.
- 1300 Werner, C., Schnyder, H., Cuntz, M., Keitel, C., Zeeman, M.J., Dawson, T.E., Badeck, F.-W., Brugnoli,
1301 E., Ghashghaie, J., Grams, T.E.E., Kayler, Z.E., Latatos, M., Lee, X., Maguas, C., Ogee, J.,
1302 Rascher, K.G., Siegwolf, R.T.W., Unger, S. & Werner, C. (2012) Progress and challenges in

- 1303 using stable isotopes to trace plant carbon and water relations across scales. *Biogeosciences*, **9**,
 1304 3083-3111.
- 1305 Werner, C., Zumkier, U., Beyschlag, W. & Maguas, C. (2010) High competitiveness of a resource
 1306 demanding invasive *Acacia* under low resource supply. *Plant Ecology*, **206**, 83-96.
- 1307 Wessels, F.J., Jordan, D.C. & Hahn, D.A. (2010) Allocation from capital and income sources to
 1308 reproduction shift from first to second clutch in the flesh fly, *Sarcophaga crassipalpis*. *Journal of*
 1309 *Insect Physiology*, **56**, 1269-1274.
- 1310 West, A.G., February, E.C. & Bowen, G.J. (2014) Spatial analyses of hydrogen and oxygen stable
 1311 isotopes ("isoscapes") in ground water and tap water across South Africa. *J Geol Explor*, **145**, 213-
 1312 222.
- 1313 West, J.B., Bowen, G.J., Dawson, T.E. & Tu, K.P. (2010) Understanding Movement, Pattern, and Process
 1314 on Earth Through Isotope Mapping pp. 487. Springer Science and Business Media, Dordrecht,
 1315 Netherlands.
- 1316 West, J.B., Sobek, A. & Ehleringer, J.R. (2008) A simplified GIS approach to modeling global leaf water
 1317 isoscapes. *PlosOne*, **3**, e2447.
- 1318 Westphal, M.I., Browne, M., MacKinnon, K. & Noble, I. (2007) The link between international trade and
 1319 the global distribution of invasive alien species. *Biol Invasions*, **10**, 391-398.
- 1320 Whelan, T., Sackett, W. & Benedict, C. (1973) Enzymatic fractionation of carbon isotopes by
 1321 phosphoenolpyruvate carboxylase from C₄ plants. *Plant Physiology*, **51**, 1051-1054.
- 1322 Wilson, J.R., Dormontt, E.E., Prentis, P.J., Lowe, A.J. & Richardson, D.M. (2009) Something in the way
 1323 you move: dispersal pathways affect invasion success. *Trends Ecol Evol*, **24**, 136-144.
- 1324 Woodford, D.J., Richardson, D.M., MacIsaac, H.J., Mandrak, N.E., van Wilgen, B.W., Wilson, J.R. &
 1325 Weyl, O.L.F. (2016) Confronting the wicked problem of managing biological invasions.
 1326 *Neobiota*, **31**, 63-86.
- 1327 Yang, W., Jeelani, N., Leng, X., Cheng, X. & An, S. (2016) *Spartina alterniflora* invasion alters soil
 1328 microbial community composition and microbial respiration following invasion chronosequence
 1329 in a coastal wetland of China. *Science Reports*, **6**, 26880.
- 1330 Yeakel, J.D., Patterson, B.D., Fox-Dobbs, K., Okumura, M.M., Cerling, T.E., Moore, J.W., Koch, P.L. &
 1331 Dominy, N.J. (2009) Cooperation and individuality among man-eating lions. *PNAS*, **106**, 19040-
 1332 19043.
- 1333 Yelenik, S.G. & D'Antonio, C.M. (2013) Self-reinforcing impacts of plant invasions change over time.
 1334 *Nature*, **503**, 517.
- 1335 Zhang, W., Hendrix, P.F., Snyder, B.A., Molina, M., Li, J., Rao, X. & Siemann, E.F., S. (2010) Dietary
 1336 flexibility aids Asian earthworms invasion in North American forests. *Ecology*, **91**, 2070-2079.
 1337

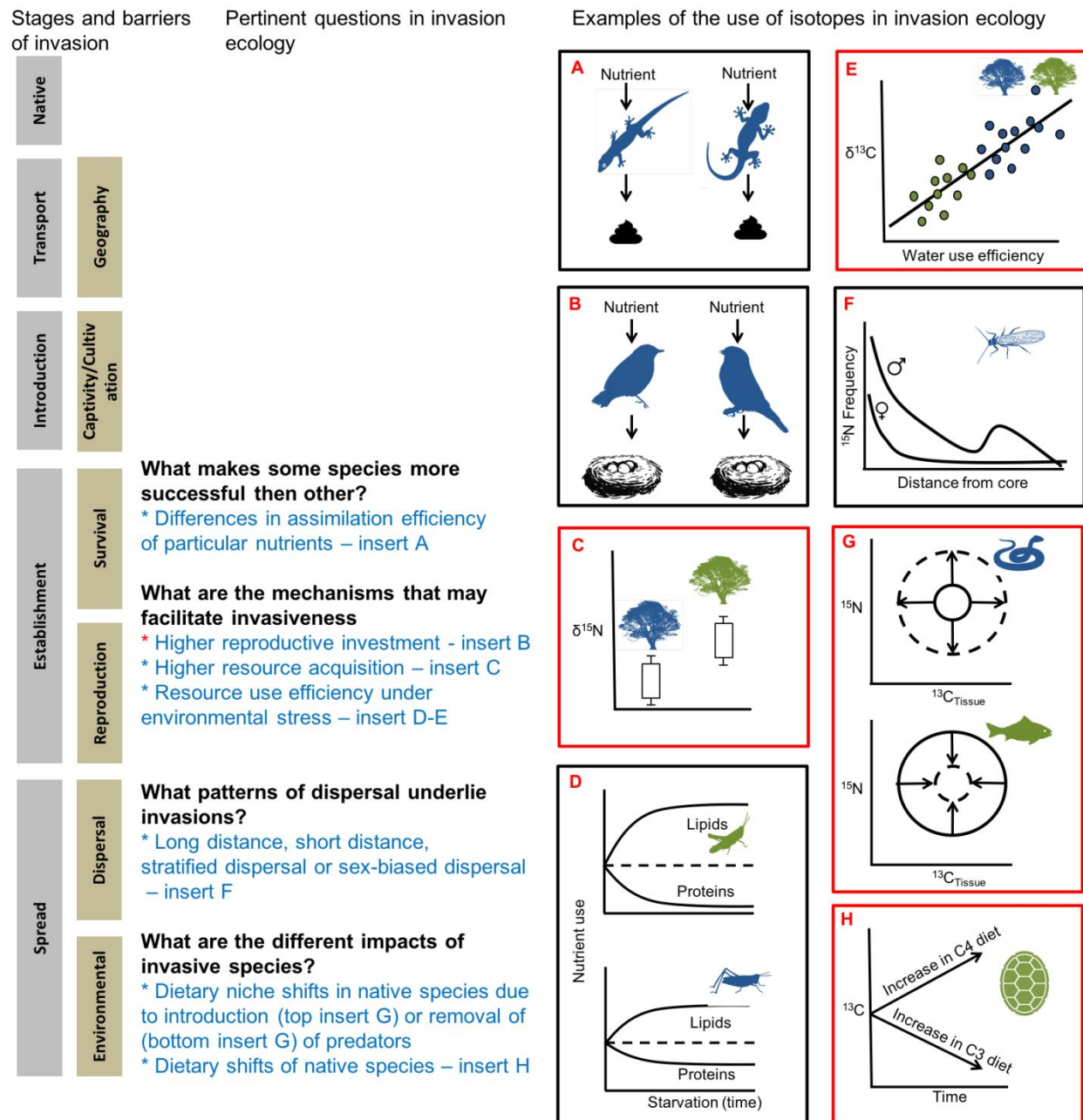
Figure 1

Figure 1: Main research questions pertaining to how introduced species progress along the introduction-naturalization-invasion continuum are stated and the role that stable isotope analyses may play in answering these are depicted as hypothetical examples. Note that most taxon comparisons are between native (green) and invasive (blue) taxa (e.g. inserts C-E) but that most approaches can also be applied

8 between different invasive taxa (e.g. insert A-B) to assess differential invasiveness, impacts, etc.
9 Examples of mechanisms that utilize natural isotopes are shown in red boxes and those making use of
10 artificially enriched isotopes in black boxes.

11

For Review Only