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Assessment of Animal-Based Methods Used for Estimating and Monitoring Rangeland Herbivore Diet Composition☆

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ABSTRACT

Researchers and managers need effective tools for monitoring the use of forages by large herbivores. Since 2000, the number of herbivore diet studies has nearly doubled. In this review, we determine trends in the field; assess the utility of key techniques against five criteria (cost, accuracy and precision, resolution, utility for long-term monitoring programs, and appropriateness for browsers and grazers); and make recommendations to give managers appropriate tools. Three techniques stand out: microhistology, near infrared reflectance spectroscopy, and deoxyribonucleic acid (DNA) barcoding. Microhistology has a long history of use in rangelands and is often considered the gold standard for understanding diet composition, albeit at a high cost of labor. Near infrared reflectance spectroscopy can resolve the presence of target groups or species more quickly than microhistology, especially for grazers. DNA barcoding provides the greatest resolution of dietary items with less quantitative certainty than microhistology. The costs associated with DNA barcoding come primarily from technology and sequencing, while in microhistology they are associated with labor. Therefore, an improved, streamlined microhistology method could provide rangeland managers a rapid and cost-effective method for diet monitoring. Ultimately, the complex challenges facing rangeland managers today may require the use of more than one method to achieve acceptable resolution within actionable time frames.

Introduction

Herbivorous mammals play a key role in the structure and function of rangelands (Hobbs, 1996), particularly in systems where predators are functionally absent (Vavra et al., 2007) and where native species interact with domestic livestock and exotic or feral herbivores (Schwartz and Ellis, 1981; Bakker et al., 2006; Vavra et al., 2007; Nuñez et al., 2010).The impacts of mammalian herbivores on rangelands depend on both environmental features such as aridity and history of disturbance and animal features including body size, diet type, and evolutionary origin (native/exotic/domestic) (Augustine and McNaughton, 1998; Bakker et al., 2006; Vavra et al., 2007). Diet is particularly important, as it gives insight into ecological and evolutionary processes like habitat selection, competitive interactions (e.g., coevolution of primary producers), and body condition in the typically nutrient poor environment of rangelands (Krebs, 1998). Forage for large herbivores consists primarily of graminoids (monocotyledons such as grasses and sedges), forbs (herbaceous dicotyledons), and browse (woody dicotyledons), with fruits, fungi, and seeds making up smaller components of the diet (du Toit and Olff, 2014).

Understanding and managing the complex relationships between herbivores and plants in rangelands requires effective techniques for monitoring herbivore diet. As all communities experience temporal change, one of the biggest challenges for monitoring programs is to distinguish among factors (e.g., natural climate change vs. over stocking) (Magurran et al., 2010). For this reason, long-term monitoring, ideally across 20- to 30-yr time frames, is desirable for making decisions about landscape management practices such as habitat modification through prescribed burning, managed stocking densities, and harvest regimes.

Methods to assess herbivore diet focus on measuring either the amount of plants that have been removed (plant-based methods) or physical/ chemical aspects of samples collected from animals (animal-based methods). Plant-based methods are of limited use for free-ranging animals, so they will not be discussed further here (Holechek et al., 1982; Mayes and Dove, 2000). Animal-based methods to assess diet composition in free-ranging mammalian herbivores range from

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direct observation of feeding activity to measures of markers and metabolites from the diet in feces (Holechek et al., 1982; Mayes and Dove, 2000; Barboza et al., 2009). Reported diets are affected by the method, material analyzed, and their interaction. Appropriate methodology may differ depending on whether the herbivore is domestic or wild, as the diets of domestic livestock can be more readily sampled by observation and invasive procedures (e.g., fistulas). For long-term monitoring programs, sampling should be noninvasive, as this allows for repeated measurements and is appropriate for common and rare species. Fecal collection is simple, repeatable, inexpensive, and applicable to both wild and domestic species. However, all techniques that rely on postingestive samples are subject to the effects of differential digestion (e.g., rates of flow and extraction of nutrients) of plant species and plant parts (Fig. 1). Increased fragmentation and breakdown of soft components (e.g., leaves), compared with harder components (e.g., stems) all impact the accuracy and precision of the diet estimate over and above any sources of error inherent in particular techniques.

Comprehensive reviews of techniques for estimating herbivore diets were published by Holechek and colleagues in 1982 and again by Mayes and Dove in 2000. Recent advances in technology, particularly the advent of deoxyribonucleic acid (DNA) barcoding, have increased the options available to managers to assess herbivore diets. It is therefore appropriate to revisit techniques for herbivore diet measurement and to make recommendations about timely and effective methods for answering key management questions.

In this review we:

- Survey the techniques used in recently published literature (since 2000), to determine trends in the field;
- Assess the utility of these key techniques for current applications against five criteria (cost, accuracy and precision, resolution, utility for long-term monitoring programs, and appropriateness for browsers and grazers); and
- Make recommendations to give managers appropriate tools for timely diet assessment.

Survey of Techniques

We focused our review on commonly used animal-based methods to assess free-ranging herbivore diets: behavioral observation, microhistology, near infrared reflectance spectrometry (NIRS), stable isotopes, cuticular wax alkanes, and DNA barcoding. Using Web of Science (an online database containing information from ≈8 500 research journals worldwide), we searched for papers published between 2000 and 2017 with the words "herbivore" and "diet." We only included records within documents classified as articles, data papers, database reviews, discussions, early access, or proceedings papers. Our search in February 2018 yielded 2 981 results. On the basis of the inspection of the top results, as well as our understanding of the field, we narrowed our search by including one of "bite count OR bite rate," "microhistology," "NIRS," "n-alkane AND wax," "stable isotope," or "DNA barcode" We also included either "selection" or "composition" to try to eliminate papers that looked solely at diet quality or digestibility. We excluded "insect," "reptile," "bird," "fish," "marine," or "carnivore" because we were interested in the use of techniques based on plant fragments. There were 417 papers included in our final survey.

There were almost twice as many papers published on herbivore diet in 2015 − 2017 ($n = 80$) as there were in 2000 − 2002 ($n = 45$). The relative use of behavioral observation and microhistology has declined slightly in the past few years, while NIRS and stable isotope methods have remained relatively consistent (Fig. 2). Use of cuticular

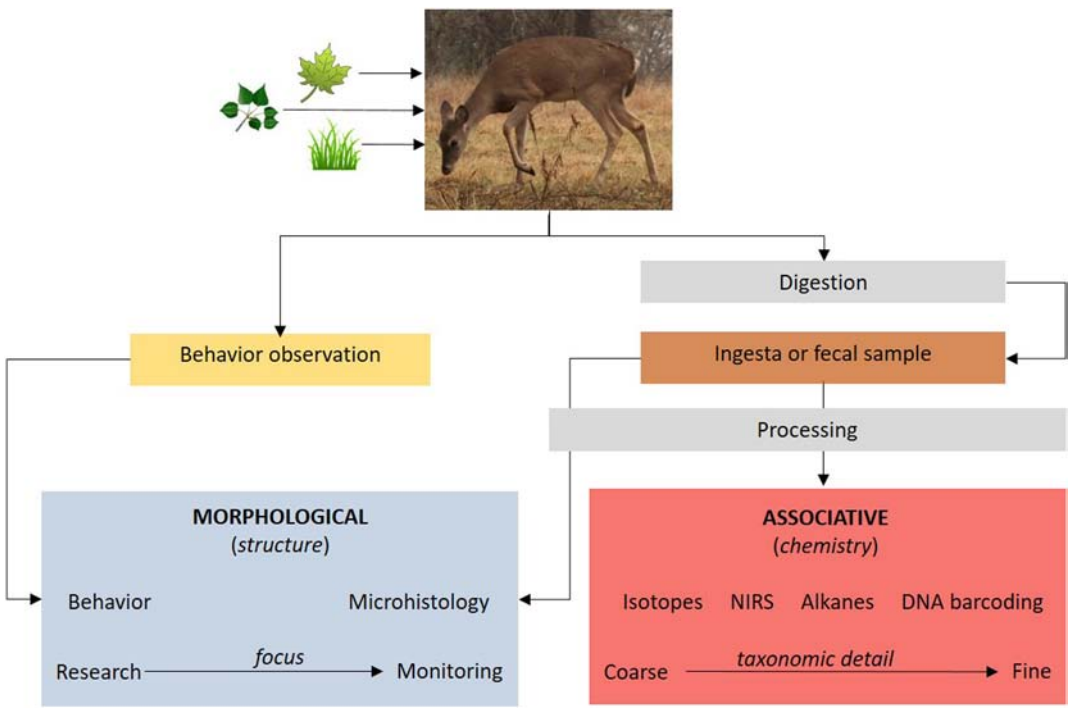

Figure 1. Noninvasive, animal-based techniques to assess free-ranging mammalian herbivore diet composition. Morphological techniques identify diet components based on structural characteristics of plants. Behavioral observation is the only technique that does not involve digestion of a sample by the herbivore before observation by the researcher or processing by the researcher before analysis. This means that this technique is uniquely unaffected by digestion of diet components. Due to the time involved in behavioral assessment, this technique is best suited to research questions. Microhistology is equally suited to research and monitoring. Associative techniques range in scale from chemically assessing elements (isotope analysis) to chemically assessing sequences (deoxyribonucleic acid [DNA] barcoding). Isotopic analysis gives the coarsest taxonomic scale (grass vs. browse), whereas DNA barcoding can identify plant subspecies in some applications.

wax n-alkanes has noticeably declined since about 2009. In the same time period, the use of DNA barcoding has greatly increased.

Assessment Criteria

Forages are identified by morphological characteristics in behavioral observation and microhistology, whereas the chemical properties of samples are used to reconstruct diets in stable isotope analysis, NIRS, cuticular wax alkanes, and DNA barcoding (see Fig. 1). We separately evaluated each technique against five criteria: cost, precision, resolution, timescale, and diet type. We compare the techniques in the conclusion of our paper.

Cost

The cost of monitoring includes setup costs, longer-term running costs, and personnel time and effort. These costs can be prohibitively high in some cases, especially in developing countries (e.g., Caro, 2016). While some authors explicitly compare costs between techniques (e.g., Newmaster et al., 2013), we focus instead on the relative contribution of labor versus equipment to the expense of particular techniques. This is because the costs associated with each technique depend greatly on the level of replication (i.e., number of samples) and resolution (e.g., conducting microhistology on functional groups vs. individual plant species). Large sample size reduces the cost for techniques with high setup costs and low labor costs for individual samples (e.g., NIRS) but not techniques that have high labor cost per sample (e.g., behavioral observation). Lower resolution can decrease the cost for microhistological analysis because less time is spent identifying individual species, but reduced resolution is not an option for some techniques (e.g., stable isotope analysis). Decisions about the tradeoff between replication and resolution depend critically on the objectives of particular studies.

Accuracy and Precision

Precision refers to the repeatability of a technique when performed on the same sample. Accuracy is the similarity between the estimated diet and actual diet consumed by the herbivore (Figs. S1, S2 [available online at https://doi.org/10.1016/j.rama.2018.03.003) and is much harder to assess, especially when precision is low or the actual amount of the diet item is low. All techniques will have some tradeoffs among cost, labor input, accuracy, and precision (Amos et al., 2014).

Resolution

Resolution refers to the taxonomic level (e.g., family, genus, species) that a technique is capable of detecting and affects the level of change required before a signal can be detected. Some methods are only capable of determining differences in browse versus graze consumption (Mayes and Dove, 2000), while others may detect plant identity down to the subspecies level (Soininen et al., 2009). Variation is higher at lower taxonomic ranks and requires more intensive sampling to detect differences. As diet diversity increases, the number of minor species increases and the ability to detect changes in proportions of minor components declines. While low abundance items can be important (e.g., for nutritional balancing: Raubenheimer and Simpson, 1997), changes in the signals of these items are typically hard to detect (but see Altangerel et al., 2017). It is more likely that monitoring will pick up long-term shifts in the bulk of the diet of graminoids or browse.

Timescale

Ideally, long-term monitoring programs use a single, unchanged technique for data collection (Magurran et al., 2010). Although changes to methodology can be incorporated (e.g., Freeman et al., 2007), analysis of data is simpler when methodological variation is minimized. The stability of methods over time is an important determinant of their utility in long-term monitoring. This criterion should not count against innovation (Magurran et al., 2010), but it urges caution in using new, relatively untried techniques in establishing monitoring programs.

Diet Type

Herbivores tend to specialize as browsers or grazers to best contend with the challenges associated with particular forage types (Hofmann and Stewart, 1972). Browsers have morphological and phyisiological adaptations to avoid or tolerate plant chemical defenses and to digest highly lignified cell walls of dicotyledonous plants. Conversely, grazers are adapted for longer retention of plant material to increase digestion of less lignified cell walls of monocotyledonous plants (Clauss et al., 2008). Grazers are better studied than browsers because domestic livestock are mostly grazers (sheep, cattle, and horses), and only domestic goats are browsers (du Toit and Olff, 2014). Compared with grazers, the diets of browsers tend to contain more species and a wider range of plant secondary compounds, which complicate some analyses.

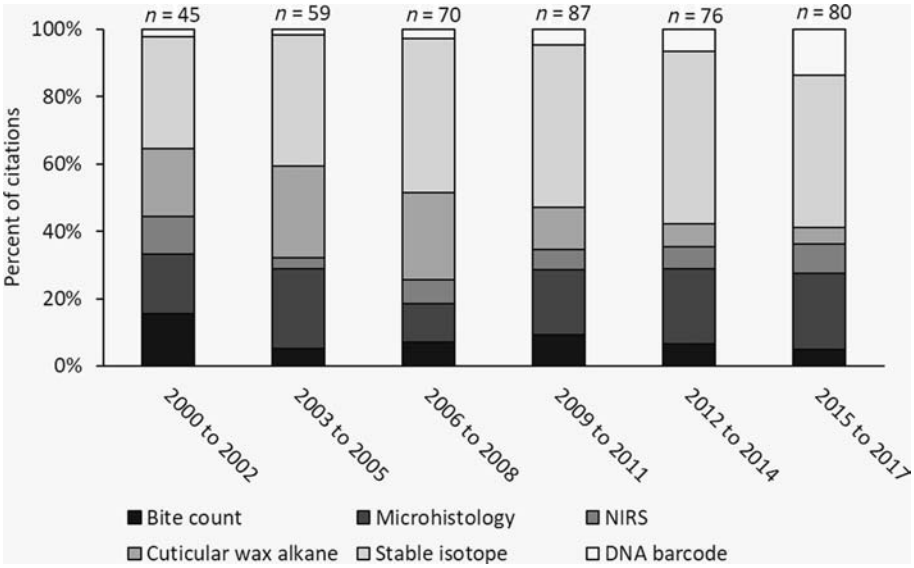

Figure 2. Relative use of techniques to assess herbivore diet composition between 2000 and 2017, assessed through citations in Web of Science. Numbers above bars indicate number of citations observed in each time period.

There is a need to develop rapid and reliable techniques to assess browser diets.

Behavioral Observation

Behavioral observation has long been used to determine diet composition in livestock (Cory, 1927) and wildlife (Dixon, 1934; Wallmo and Neff, 1970). In its simplest form, the technique requires little equipment and is straightforward to implement. Recently, animal-borne video recording systems show promise for capturing behavioral data such as diet selection without the need for direct observation. First used on turtles in 1987 (Moll et al., 2007), these systems have been modified for use in ungulates (Beringer et al., 2007) and are reasonably efficient at capturing data on foraging behavior. For example, Newmaster et al. (2013) found a high correlation (70%) between plant groups in the diet of woodland caribou *(Rangifer tarandus caribou)* estimated by video recording and DNA barcoding.

Diet selection is directly observed, so there is no processing of the diet sample by the animal or the researcher (see Fig. 1). This eliminates errors such as differential digestibilities that plague techniques based on fecal samples (e.g., Shrestha and Wegge, 2006). Even with extensive training, though, it may be impossible to identify some plant species from a distance. Direct observation of behavior requires little equipment, but the cost of equipment may be offset by the large commitment of labor and limited number of animals visible to each observer. Labor costs are lower for indirect observation of behavior with recorders, but the systems can be expensive to acquire and maintain (e.g., Newmaster et al., 2013). Due to the time-consuming nature of observations, behavioral observation studies typically have limited sample size, particularly those using animal-borne video (e.g., Beringer et al., 2007, $n = 3$; Thompson et al., 2012, $n = 5$; Newmaster et al., 2013, $n = 15$). Functional groups, such as grasses/forbs/shrubs and trees can easily be identified, and with appropriate training, it is possible to identify diet items to species or genus level. Currently, video recording systems are not adequate to distinguish plant species (e.g., Newmaster et al., 2013).

Provided that sampling protocols are well documented, a condition that applies equally to all techniques, direct observation of behavior is well suited to long-term monitoring and has a long history of use. Other advantages include the ability to simultaneously collect data on animal condition, habitat use, behavioral interactions, and activity budgets, all of which can help influence management decisions (Holechek et al., 1982).

The key disadvantage of this technique is its inapplicability to situations where animals cannot be directly observed, such as for rare and cryptic species or visually dissected habitats. At the landscape scale, such as in a savanna, this limitation applies disproportionately to browsing species, as grazers typically forage in open habitats. At the bite scale, it is easier to observe selection of aboveground browse than surface-level grasses. Behavioral observation is therefore more viable for use in livestock, as they are more readily observed (Shrestha and Wegge, 2006; Damiran et al., 2013). One alternative is to use habituated or tame wildlife (Wallmo and Neff, 1970; Timmons et al., 2010). However, because tamed animals are normally captured shortly after birth with limited opportunity to experience the socially facilitated learning component of diet selection, the assumption that these animals are representative of the broader population should be confirmed (Holechek et al., 1982) by comparing diets of habituated and wild animals (Spalinger et al., 1997). Here animal-borne video shows promise as a technique to augment other data capture systems (e.g., Global Positioning System telemetry, Thompson et al., 2012), particularly for species that are challenging to observe (Moll et al., 2007).

Microhistology

First reported by Baumgartner and Martin (1939), microhistology is a well-established and stable technique. Users identify plant cuticle fragments through characters such as cell size, shape, and arrangement using a reference collection of known plant species (Holechek et al., 1982; Norbury, 1988). Recently, some innovative approaches to machine learning and pattern recognition have capitalized on the capacity for computers to recognize and identify images to help solve ecological questions (e.g., Bolger et al., 2012; Yu et al., 2013; Swinnen et al., 2014). While we are unaware of any attempts to do so, such methods could reasonably be applied to reading microhistology slides of herbivore diet to improve the accuracy, precision, and speed and reduce expenses.

Because it uses simple equipment that is widely available, setting up microhistology is relatively inexpensive (Holechek et al., 1982; Mayes and Dove, 2000). However, microhistology is time intensive and it may take users a substantial amount of time to become proficient in the technique (Holechek and Gross, 1982). Automated image recognition processes would assist in reducing labor costs.

A time-consuming aspect of the technique involves creating and becoming familiar with the reference collection. Recent advancements in the ability to digitize and classify reference slide collections (e.g., Shrestha and Wegge, 2006) enhance the ability to transfer reference collections between labs, thereby reducing setup time for this technique but not training time for each technician. Recently, Desbiez and Santos (2014) developed an interactive, online taxonomic key for identifying microhistological characters for 206 species of plants consumed by herbivores in the Brazilian Pantanal. The defining feature of interactive keys is that characters can be used in any order; characters that are not available in a particular epidermal fragment on a slide, or whose interpretation is not clear to the user, can be avoided (http://delta-intkey.com). Such keys could be developed for other ecosystems and herbivores. Their use would greatly speed up the time for new users to learn microhistological plant identification and improve consistency between users (Desbiez and Santos, 2014).

The greatest disadvantage of microhistology is the potential effect of differential digestibilities of individual plant parts, species, or functional groups (Holechek et al., 1982; Mayes and Dove, 2000). Under such a situation, highly digestible components of the diet are likely to be underestimated (e.g., Shrestha and Wegge, 2006; Wam and Hjeljord, 2010). To improve precision, correction factors for differential digestibilities (e.g., Leslie et al., 1983; Norbury, 1988) and observer training (Holechek and Gross, 1982) have been developed. In some studies, macrohistological examination of rumen or stomach contents has been used to detect stems, seeds, fungi, or flowers (Forsyth and Davis, 2011).

Microhistology can generate high-resolution information about diets, down to individual species at times (Holechek et al., 1982; Mayes and Dove, 2000). However, to minimize concerns about differential digestibilities and the number of fragments that cannot be identified, many studies report diet composition at higher levels, such as family or functional group.

As for all methods using fecal samples, microhistology is well suited to long-term monitoring programs because sample collection is relatively straightforward and repeat samples can be collected from individuals or populations. Due to its long history of use, in many cases there is a wealth of published data using microhistology against which to compare current patterns (e.g., Scasta et al., 2016). Finally, microhistology is equally suited for use in grazers and browsers.

Near Infrared Reflectance Spectroscopy

This technique is based on the principle that reflectance in the near infrared spectrum represents the chemical structure of a sample. It was first applied to understanding herbivore forage quality by Norris et al. (1976). NIRS can be conducted on fecal, ingesta, or forage samples. Applications on fecal samples are not subject to the effects of differential digestibility of diet components because calibrations are based on the relationship between fecal spectra and diets of known composition.

Fecal NIRS has most often been used to determine the botanical composition of diets by estimating the presence of one (e.g., Walker et al., 1998, 2002; Snowder et al., 2001; Waldron et al., 2009; Jean

et al., 2014) or two focal species/genera (e.g., Glasser et al., 2008). The greatest weakness of NIRS is that the technique requires independent validation and continual monitoring of calibrations (Dixon and Coates, 2009), which has rarely been done. Despite this, the technique has been independently validated in several studies that showed calibrations were robust for different communities, plant species within a genus, and herbivore species (Walker et al., 1998, 2002). However, Walker et al. (2002) cautioned that fecal NIRS for determining botanical composition should only be considered interval-scale data.

Because NIRS is nondestructive and only requires a small amount of sample, it is potentially useful for long-term monitoring (e.g., Walker et al., 2013), particularly where time and funding are available to develop a detailed reference set for calibrations. Although the technique does not identify individual diet components well, it can be used to track changes in diets over time. Furthermore, multiple constituents can be simultaneously assessed (e.g., nitrogen, fiber, tannins) (Foley et al., 1998), which gives managers additional information about animal nutritional status. For example, NIRS is widely used to monitor the diets of grazers such as domestic sheep *(Ovis aries)*, cattle *(Bos taurus)* (Walker et al., 2002), and bison *(Bison bison)* (Craine et al., 2013). In contrast, NIRS is potentially more challenging to use in browsers (but see references by Walker and colleagues), due to the diversity of plant species and chemical compounds typically found in their diets.

Stable Isotope Analysis

Stable isotopes are used as markers in a wide range of organisms. While $^{13}C/^{12}C$ and $^{15}N/^{14}N$ are most frequently used (Kelly, 2000; Mayes and Dove, 2000), other isotopes have also been used (Ehleringer and Rundel, 1988). Stable isotopes were first used to assess herbivore diet by DeNiro and Epstein (1978) and Vogel (1978). Tissue (e.g., hair, bone, tooth enamel), ingesta, or fecal samples can be used.

Although isotope analysis requires expensive equipment and considerable expertise, it is provided as a standard laboratory service that can be quick and accessible. In interpreting stable isotope data, it is important to use an appropriate discrimination factor, which may significantly influence estimates of dietary composition (Barboza et al., 2009; Codron et al., 2012; Gustine et al., 2014). Furthermore, fractionation and turnover of isotopes in tissues may obscure the contributions of particular plant types if the diet varies through time (Tieszen et al., 1983; VanSomeren et al., 2017).

The greatest disadvantage of isotopic analysis is that it is unable to give species-level resolution, so it is limited to applications such as understanding diet type (grazer vs. browser) or indicating trophic position (Mayes and Dove, 2000). The ratio of ^{13}C to ^{12}C isotopes can indicate the relative proportions of dicotyledonous (C_3) and monocotyledonous (C_4) plants in the diet (Kelly, 2000). ^{15}N can indicate the trophic position of a consumer and aridity of particular habitats, and it is more useful than ^{13}C for ecosystems with few C_4 plants or a mixture of C_3 and C_4 monocots (Kelly, 2000; VanSomeren et al., 2017).

The technique has been widely used with tissues from extinct animals. As samples from feces and tissue represent different time frames, it is possible to estimate diet across a range of scales if different samples are used simultaneously (Tieszen et al., 1983). For this reason, stable isotopic analysis is a useful technique for long-term monitoring where time and funding are available to establish a reference library and detailed data on diet composition are not required.

In some situations (e.g., where there are few C_4 plants or a mixture of C_3 and C_4 monocots), multiple isotopes and markers may need to be used to distinguish browsers from grazers (Codron and Codron, 2009). Otherwise, the technique works well for both browsers and grazers.

Plant Cuticular Wax Alkanes

The indigestible waxes and alkanes of plant cuticles contain lipid-soluble compounds that can identify plant species and parts. This method was suggested by Mayes et al. (1986) and substantially reviewed by Mayes and Dove (2000). Because the technique relies on fecal samples, it is affected by differential digestibility of diet components.

Alkane measurement requires equipment for extraction and detection that is not readily available as a standard laboratory service. Furthermore, the technique requires substantial investment to develop appropriate calibrations. The principal detraction of this technique is the finding that the signature of individual plants and plant species changes throughout the year and between locations (Carnahan, 2011). For this reason, the technique is infrequently used to estimate diet composition in free-ranging animals (see Fig. 2).

Only as many diet components can be estimated as there are markers, which depends on the uniqueness of the patterns in each mixture and statistical discrimination of multiple alkane concentrations (Barboza et al., 2009). For this reason, the technique is of limited use in systems where animals consume botanically complex diets (Bugalho et al., 2004). To get around this issue, additional components of cuticular waxes, such as wax esters, long-chain fatty acids, and long-chain fatty alcohols, have been used in addition to n-alkanes with some success (e.g., Bugalho et al., 2004; Carnahan et al., 2013).

If resources are available to develop appropriate calibration data sets, cuticular wax markers may be appropriate for longer-term monitoring. These markers also allow concurrent estimation of dietary intake (Mayes and Dove, 2000), which may provide additional data to influence management decisions. Due to limitations of use in assessing botanically complex diets (Bugalho et al., 2004) like those of browsers, the technique is best suited to grazers.

DNA Barcoding

DNA barcoding involves sequencing target DNA and matching it to a database of known sequences to identify the taxonomic origin (Valentini et al., 2009; Clare, 2014). The potential to use fecal DNA to identify diets in free-ranging animals was first suggested by Hoss et al. (1992). With the advent of next-generation sequencing, DNA analysis has become more accessible (Valentini et al., 2009; Clare, 2014) and has recently become popular for use in determining diet composition in large herbivores (e.g., Pegard et al., 2009; Ait Baamrane et al., 2012; Hibert et al., 2013; Newmaster et al., 2013; Kartzinel et al., 2015).

DNA barcoding offers fast processing and turnaround times and is provided as a standard laboratory service (Valentini et al., 2009; Clare, 2014). The costs therefore depend on the number of samples analyzed. DNA barcoding is particularly useful when the diet cannot be determined morphologically (Kohn and Wayne, 1997). Furthermore, sequences are not prone to subjective interpretation (Valentini et al., 2009; Clare, 2014). The chief advantage of the technique is that it can generate a complete list of species in the diet, down to subspecies level at times (e.g., Soininen et al., 2009), and fewer samples are required to detect a particular or rare species than other methods (Clare, 2014). It is therefore particularly useful for detecting the presence of invasive or threatened plants in herbivore diets to determine the impact of animals as vectors or consumers (Valentini et al., 2009; Clare, 2014).

Sources of error using barcoding to quantify botanical composition of diets include technological errors (e.g., amplification efficiency, extraction efficiency, bioinformatic sorting) and biological errors (e.g., tissue cell density, gene copy number, DNA survival) (Pompanon et al., 2012). DNA barcoding is a single-locus identification system (Valentini et al., 2009), and the choice of primer dictates which species will be identified (Deagle et al., 2010; Clare, 2014). Recent work on herbivore diets has focused on the chloroplast trnL intron (e.g., Taberlet et al., 2007; Pegard et al., 2009; Raye et al., 2011; Ait Baamrane et al., 2012; Hibert et al., 2013; Kartzinel et al., 2015). However, at least four common target regions have been recommended for use in plants (Clare, 2014; Kartzinel et al., 2015) as DNA barcoding of plants to species level often cannot be accomplished using a single region. In most applications, diet items can only be resolved to the family or genus

Table 1
Key limitations and best applications for noninvasive diet monitoring.

Technique	Key advantages	Key limitations	Data type[1]	Main sources of error	Best application
Behavioral observation	Little equipment Diet selection directly observed Simultaneously collect animal data	Line of sight Time consuming Small sample size	Quantitative	Observer errors in plant ID Small sample size	Shorter-term studies on readily observed animals in open habitat, where other information on behavior is desirable
Microhistology	Simple equipment Well-established technique Equally suited for browsers and grazers	Differential digestibility Time consuming Extensive reference collection and training required	Quantitative	Differential digestibility Observer error in plant ID Inadequate reference collection	Long-term monitoring using fecal samples, where effort in creating reference collection and learning identifications is worthwhile
NIRS	Rapid assessment for large number of samples Unaffected by differential digestibility Simultaneously collect nutrition data	Complex equipment Calibrations not easily transferrable Does not work well for browsers	Quantitative (limited number of species) Qualitative (most applications)	Inadequate monitoring of calibration sets Inadequate validation of calibration sets	Rapid coarse assessment where calibrations have been developed for a site, so longer-term project envisioned; or to determine if a particular plant of interest (rare or weedy) is consumed
Stable isotopes	Rapid Extinct species Simultaneously assess multiple timescales	Complex equipment Not effective in all ecosystems	Qualitative	Differential digestibility Use of inappropriate discrimination factor Fractionation/ turnover of isotopes in tissues Inadequate reference library	For coarse assessment of diet type, particularly where C_3/C_4 pathways clearly distinguish grazers/browsers
Wax alkanes/ alcohols	Simultaneously estimate intake	Complex equipment Inconsistent signatures Small number of markers Calibrations expensive to develop	Quantitative (limited number of species)	Differential digestibility Inconsistent signatures Inadequate calibration	Used as markers in digestion trials; not for assessing free-ranging diet composition with complex diets
DNA	Rapid assessment Exhaustive list of plants Where morphology cannot be distinguished Extinct species Simultaneously ID species/sex Sequences not prone to subjective interpretation	Complex equipment Over-represents rare species Single locus ID system Not easily quantitative	Quantitative (only where adequately calibrated) Qualitative (most current applications)	Differential digestibility Environmental contamination Amplification/extraction efficiency Tissue cell density/gene copy number DNA survival Imperfect reference libraries	Where an exhaustive list of all plants eaten is required; or to determine if a particular plant of interest (rare or weedy) is consumed

ID indicates identification; NIRS, near infrared reflectance spectrometry; DNA, deoxyribonucleic acid.
[1] Quantitative data on animal diets indicate the relative amounts or proportions of various plant species/functional groups in the diet. Qualitative data indicate what is consumed (e.g., functional groups, families, species) but not the contributions of these classes/items to the overall diet.

level, even with the use of additional primers (e.g., Pegard et al., 2009; Raye et al., 2011; Ait Baamrane et al., 2012; Hibert et al., 2013; Kartzinel et al., 2015).

As fecal DNA is typically degraded, it is difficult to amplify fragments longer than 150 base pairs (Valentini et al., 2009). Sequencing larger regions maximizes the taxonomic information extracted, but sample degradation affects the length that can be recovered (Clare, 2014). There may be a significant bias toward detecting undegraded DNA, which would limit taxonomic identification. Using shorter amplicons may overcome this bias but could overestimate diversity (Clare, 2014). A related concern is that heteroplasmy (the presence of multiple copies of genes), which is common in plants, can further complicate species identification (Valentini et al., 2009). There is also evidence that the mean copy number of shorter DNA fragments is higher than longer fragments, such that shorter sequences are overrepresented in the total number of sequences obtained per fecal sample (Raye et al., 2011). Finally, all DNA barcoding relies on existing databases, such as GenBank, as reference libraries. These libraries are imperfect (Harris, 2003) as a result of sequencing errors, contaminations, sample misidentifications, or taxonomic problems (Valentini et al., 2009).

While the assignment of sequences to species may be relatively straightforward, the quantitative interpretation of barcoding results is not (Valentini et al., 2009). Neither traditional quantitative genetics techniques (e.g., qPCR/rtPCR: McCracken et al., 2012), nor using the number of sequences recovered as a proxy for abundance (e.g., Deagle et al., 2010) have been particularly effective (Clare, 2014). This is because original diet items are differentially digested (fragmentation of DNA) and tissues contain different densities of DNA originally (mean copy number per diet item). These issues affect interpretations of quantity in the original diet, even in very simple systems (Deagle et al., 2010; Clare, 2014). Kartzinel et al. (2015) employed a novel approach to using relative read abundance as a proxy for proportional composition by first validating assessments of grass versus browse in the diet through stable isotope analysis. Further safeguarding against misidentification, Kartzinel et al. (2015) used multiple primers in addition to trnl to identify species and used a reference collection from their study site to compare sequence reads against. While the interpretation of DNA barcoding results is being refined (e.g., Kartzinel et al., 2015) and correction factors are being developed (e.g., Thomas et al., 2016), best practice

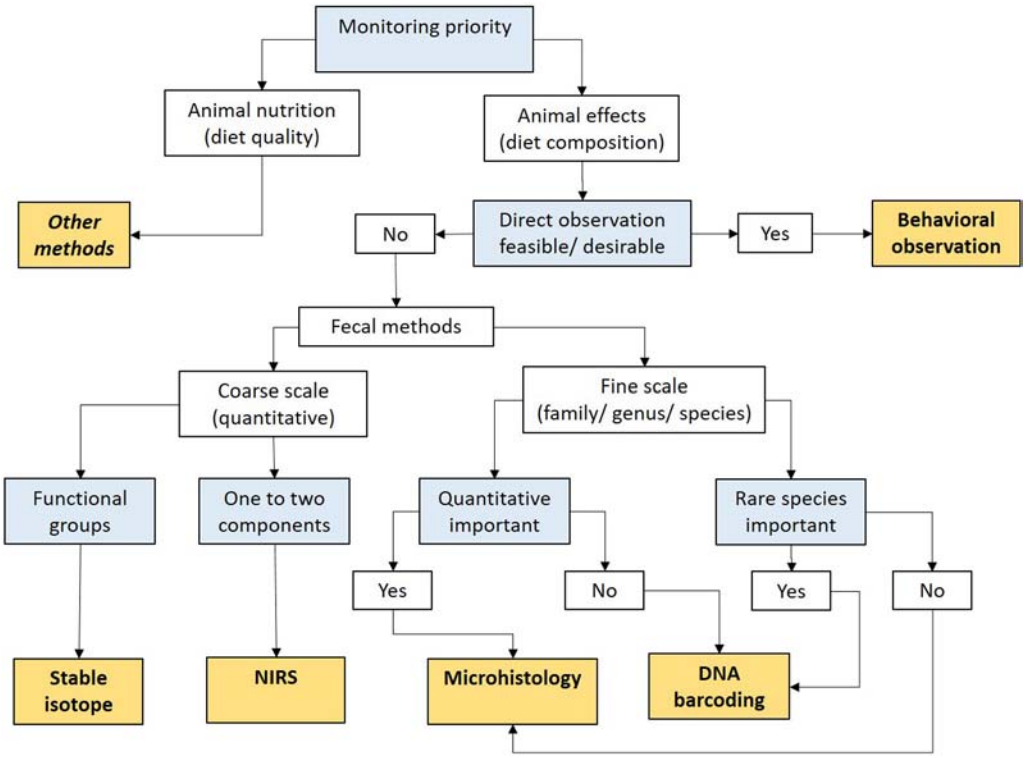

Figure 3. Decision tree for refining monitoring technique. *Blue boxes* indicate key decision points. *Orange boxes* on the edges of the figure indicate techniques. Complementary techniques may be required in any one study if multiple questions are important.

dictates that both rare and common items should simply be recorded as "present" (Pompanon et al., 2012; Clare, 2014).

DNA barcoding has been successfully used for diet analysis in extinct species (van Geel et al., 2008; Welker et al., 2014). Endogenous DNA in feces can also be used to identify or confirm contributing animal species, sex, or even individual identity (Kohn and Wayne, 1997; Raye et al., 2011). However, because DNA barcoding is a rapidly developing technique, changes in the methodology, particularly in the use of primers, mean that current diet estimates may not be comparable with those generated in the future under different methodologies. The technique is equally suited for use in browsers and grazers.

Management Implications

The best technique to assess herbivore diet depends on the monitoring priority (Table 1, Fig. 3). Therefore, well-executed monitoring programs need to have a clearly defined question (Nielsen et al., 2017). There will often be a tradeoff between cost and sample size for adequate resolution, precision, and accuracy of the system. Morphological methods (behavioral observation and microhistology, see Fig. 1) require less equipment but more labor. Associative methods (NIRS, stable isotopes, wax alkanes, and DNA barcoding) require expensive technology to process samples, but the provision of many of these techniques as standard laboratory services reduces the cost per sample and allows for quick turnarounds (Foley et al., 1998). Morphological methods potentially over-represent resource overlap between consumers by failing to detect subtle differences between plant species; molecular methods potentially over-represent rare species and overemphasize resource partitioning (Clare, 2014).

For studies that require data on diet composition, microhistology and DNA barcoding present the best options (see Fig. 3). Despite the well-known challenges, microhistology is often seen as the "gold standard" for diet assessment and is often used as reference technique against which newer techniques are compared (e.g., Soininen et al., 2009; Carnahan, 2011; Newmaster et al., 2013). If the potential for image recognition is realized for microhistology, it could become the method of choice to influence decision making about vegetation management (e.g., fire fuels) and herbivore management (e.g., harvest quotas) in a time-sensitive manner because of the potential for rapid high-resolution determination of diet composition that could be routinely validated by trained technicians. DNA barcoding is a relatively new yet increasingly popular technique (see Fig. 2). While refinements are being continuously developed (e.g., Kartzinel et al., 2015; Thomas et al., 2016), until some of the more serious challenges have been resolved, the results of DNA barcoding for diet studies in herbivores should be interpreted conservatively. Behavioral observation may be useful for some studies requiring diet composition data but is limited to situations where animals can be readily observed (see Fig. 3). If a coarser scale of measurement is appropriate, and there are resources available to develop calibration data sets, cuticular wax alkanes, NIRS, and stable isotopes are potential options that can provide rapid results (see Table 1). In particular, for studies that require botanical composition of one or two species of interest, NIRS is the most viable alternative. Ultimately, the complex challenges facing rangeland managers today may require the use of complementary techniques to achieve acceptable resolution within actionable time frames.

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Appendix A. Supplementary data

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