SELECCIÓN DE SEMILLAS Y DIETA INVERNAL DE AVES DE PASTIZAL EN EL NORTE DE MÉXICO

POR:

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RESUMEN GENERAL

SELECCIÓN DE SEMILLAS Y DIETA INVERNAL DE AVES DE PASTIZAL EN EL NORTE DE MÉXICO

POR:

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Presidente: Ph. D. Alicia Melgoza Castillo

Las poblaciones de aves de pastizal han disminuido severamente en la última década. La razón principal es la pérdida de hábitat, pero falta información acerca de la distribución de recursos y disponibilidad y diversidad de semillas sobre la sobrevivencia de gorriones. En este trabajo se estudió la dieta invernal de *Ammodramus bairdii* y *A. savannarum* en diferentes sitios del desierto Chihuahuense y su habilidad para aceptar semillas de pastos introducidos. Primeramente, se realizó un trabajo piloto con gorrión Inglés (*Passer domesticus*) para obtener experiencia y establecer el experimento sobre preferencia. Las preferencias de semillas se relacionaron con características de las mismas como tamaño, color, visibilidad y nutrientes. De estas características solo el tamaño fue importante. En un segundo estudio, se evaluó la preferencia de semillas de pastos nativos e introducidos con tres gorriones silvestres (*A. bairdii*, *A. savannarum* y *Passerculus sandwichensis*). De las especies introducidas, sólo fueron consumidas las semillas de *Melinis repens*, pero no las de *Eragrostis* *lehmanniana* y *Pennisetum ciliare*. Estas especies de pastos introducidos pudieran representar una amenaza para las aves invernales. Las preferencias fueron influidas por la eficiencia de manipulación. En un tercer estudio se tomaron muestras estomacales de gorriones capturados en pastizales. Los resultados muestran que, bajo condiciones naturales, *Ammodramus* spp. son selectivas y no consumen semillas en proporción a su disponibilidad. Las semillas preferidas pertenecen a los grupos Panicoideae; además de *Verbena* y *Pleurpahis mutica*. Las semillas preferidas no siempre fueron las semillas más comunes en la dieta; las semillas de varias especies de *Bouteloua* fueron las segundas más comunes en la dieta. Los resultados indican la importancia de conservar zacates nativos en el hábitat de las aves de pastizal y en particular, de favorecer la producción de semillas de Panicoideae y *Bouteloua* spp. en el hábitat de *Ammodramus* spp.

ABSTRACT

SEED SELECTION AND WINTER DIET OF GRASSLAND BIRDS IN NORTHERN MEXICO

BY:

MIEKE TITULAER

Grassland bird populations have undergone sharp declines over the past decade. The main reason for these population declines is probably habitat loss, but little is known about the influence of winter habitat quality, resource distribution and seed availability and diversity on sparrow survival. Here, the winter diet of Baird's Sparrow (*Ammodramus bairdii*) and Grasshopper Sparrow (*A. savannarum*) was studied across different sites in the Chihuahuan Desert, as well as the ability of these sparrows to accept introduced grass seeds. First, a pilot study was performed with House Sparrows (*Passer domesticus*) to gain experience with seed preference experiments. Seed preferences were related to seed characteristics such as size, color, visibility and nutrient content. Of these characteristics, only seed size was important in determining selectivity of House Sparrows. Next, seed preferences for native and introduced seeds were evaluated in three wild sparrows (*A. bairdii*, *A. savannarum* and *Passerculus sandwichensis*). Sparrows were able to accept seeds from the introduced *Melinis repens*, but *Eragrostis lehmanniana* and *Pennisetum ciliare* were avoided, indicating that these grasses could be a threat to over-wintering sparrows. Seed preferences for both native and introduced seeds were influenced by seed size and handling efficiency. Finally, regurgitated stomach samples from free living sparrows indicate that, under natural conditions, *Ammodramus* spp. are selective and do not consume seeds in proportion to their availability. Preferred seeds belonged to Panicoideae, and in some sites *Verbena* spp. and *Pleuraphis mutica*. Preferred seeds were not always the most common seeds in the diet; *Bouteloua* spp. were the second most common seeds in the diet. The results indicate the importance of conserving native grasses in grassland bird habitat. Grassland management practices should, in particular, favor seed production of Panicoideae and *Bouteloua* spp. in the habitat of *Ammodramus* spp.

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INTRODUCTION

Grassland birds form an important group of conservation concern in North America. Grasslands are widely recognized as the most threatened terrestrial ecosystem and the loss of suitable winter habitat is likely the most important cause of declining grassland bird populations (Askins, 2007). Previous research has demonstrated the importance of grassland habitat structure in influencing both the winter abundance and survival of small granivorous grassland bird species (Macías-Duarte *et al*., 2009). However, food availability and diet are also important factors influencing habitat suitability. For example, nutritional requirements and seed handling efficiency of different seed species likely influence which habitats are adequate for the survival of a particular bird species, but knowledge of the relative importance of grasses and other plant seeds in the winter diets of grassland birds is lacking. Insights in habitat requirements and diet are important to know which plant species should be promoted in grassland management and restoration efforts, in addition to prioritizing grassland types for protection. Furthermore, this information will help to guide and evaluate grassland improvement projects and ensure that limited resources are used most effectively.

The objective of this project was to investigate the diet composition of granivorous sparrows overwintering in the desert grasslands of northern Mexico. The focus was on two bird species in particular, Baird's Sparrow (*Ammodramus bairdii*) and Grasshopper Sparrow (*Ammodramus savannarum*). The knowledge on the diet of these two species will help to inform management plans for the protection of these and other grassland passerines. A second objective was to investigate the mechanism by which birds select their diet. This is important to understand why particular seeds are preferred or rejected and to formulate hypotheses and predictions for the future, for example about what would happen when the bird's habitat is invaded by exotic grass species.

The present work is divided in three studies. The first sudy describes the results of a pilot study with House Sparrows (*Passer domesticus*) in which the importance of different seed characteristics and nutritional components was investigated. The second study is a seed preference experiment performed with wild sparrows in captivity, in which preferences for native and introduced seed species were compared to see whether birds are able to accept seeds from introduced grass species that are common in northern Mexico. The final study describes a field study in which the diets of free living *Ammodramus* spp. were investigated in different sites across the Chihuahuan desert in northern Mexico to gain insight into which grass species are important in nature.

LITERATURE REVIEW

Threats to Grassland Ecosystems and their Birds

Grasslands in North America are one of the most threatened terrestrial ecosystems in the world (Manzano-Fisher *et al*., 2006; Ceballos *et al*., 2010). The biggest threats to grassland ecosystems involve conversion to cropland, fragmentation, overgrazing, climate change, and inappropriate fire management (Pidgeon *et al*., 2001; Ceballos *et al*., 2005; Stoleson *et al*., 2005; Jetz *et al*., 2007; Curtin *et al*., 2008; Pool *et al*., 2014). These human-related events cause desertification, shrub encroachment, severe soil erosion and changes in vegetation cover and loss of important grass species (Curtin *et al*., 2002; Ceballos *et al*., 2010; Steidl *et al.*, 2013).

Over-wintering grassland birds. The change and decline of native grassland areas has detrimental results for populations of vertebrates and invertebrates that depend on these ecosystems for their survival such as desert grassland birds (Desmond, 2004; Seigel *et al.*, 2010). Because of this, Brennan and Kuvlesky (2012) refer to grassland bird declines as a conservation crisis. More than 30 migratory bird species breed in the United States (U.S.A.) and over-winter in the Chihuahuan Desert grasslands of the south-western U.S.A. and northern Mexico. Population data from the North American Breeding Bird Survey and the Christmas Bird Count show that grassland birds have declined more than any other group of birds (Butcher and Niven, 2007). More than 80 % of all grassland bird species have shown a decline in their populations since 1966 (Sauer, 2008). A common factor among these migratory birds is their dependence on and concentration in desert grasslands in northern Mexico and south-western

U.S.A. in winter (Poole, 2012). Reduced winter survival, due to reduction of suitable habitat for overwintering, is therefore expected to be an important causal factor in the decline of migratory grassland bird populations (Vickery and Herkert, 2001; Beyer and Panjabi, 2010; Macías-Duarte and Panjabi, 2013a). In order to protect grassland bird populations from extinction, it is essential to conserve, enhance and restore desert grasslands throughout the Chihuahuan Desert.

Baird's Sparrow and Grasshopper Sparrow. Some grassland species, such as Baird's Sparrow and Grasshopper Sparrow, have declined by as much as 70-90 % since 1966 (Sauer *et al*., 2011). Because of these declines both species are listed by the U.S. Fish and Wildlife Service as Birds of Conservation Concern (USFWS, 2008). Baird's Sparrow is also listed as a species of high tri-national concern by Partners in Flight (PIF), a partnership among Canada, U.S.A., and Mexico aiming to conserve shared bird species (Berlanga *et al.,* 2010). Therefore, it is important to gain knowledge on the habitat requirements of these species, in order to be able to protect them.

Baird's Sparrow and Grasshopper Sparrow belong to the order Passeriformes, family Emberizidae and genus *Ammodramus* (Vickery, 1996; Green *et al.*, 2002). Both species are grassland obligates which means they depend on grassland ecosystems for their survival (Panjabi *et al*., 2010). The average weight of a Baird's Sparrow is about 19 g for males and 17.8 g for females. Total length is on average 12 cm with a mean exposed culmen length of 10.7 mm for males and 10.4 mm for females (Green *et al.*, 2002). Grasshopper sparrows weigh between 14.5 – 20.0 g and have a total length of 10.8 – 11.5 cm (Vickery, 1996). The bill lengths of males and females are on average 11.9 mm and 10.9 mm, respectively (Crossman, 1989 in Vickery, 1996). Baird's Sparrow and Grasshopper Sparrow are native to southern Canada and northern U.S.A. In winter Baird's Sparrow migrates to Arizona, New Mexico and Texas in the U.S.A. and Chihuahua, Sonora, Durango and Coahuila in Mexico (Green, 2002), whereas Grasshopper Sparrow migrates to southern U.S.A. and all Mexico (Vickery, 1996). Within both summer and winter areas, Grasshopper Sparrows are more numerous and widespread than Baird's Sparrows, and there is a great variability in the abundance of both species between different grassland areas (Macías-Duarte *et al*., 2010).

Habitat Characteristics

Vegetation. One factor related to variability in distribution and abundance is variation in habitat characteristics (Macías-Duarte *et al*., 2009). Both Baird's Sparrow and Grasshopper Sparrow forage solitary and have been positively associated with dense grass cover, grass height, herbage cover and limited shrub cover (Pulliam, 1983; Gordon, 2000; Macías-Duarte *et al*., 2009; Martínez-Guerrero *et al*., 2011; 2014), and absence of bare ground cover (Henderson and Davis, 2014). A reduction of woody cover generally increases abundance of grassland birds (Block and Morrison, 2010). However, woody vegetation may also provide protection and most species are able to tolerate some amount of woody cover (Igl and Ballard, 1999). It is possible that habitat selection is a trade-off between foraging efficiency and predation pressure, in the sense that shrub cover offers more protection against predators whereas shorter vegetation and limited shrubs facilitate foraging efficiency (Macías-Duarte and Panjabi, 2013b). Baird's and Grasshopper Sparrows are also sensitive to the size of an area (Ribic *et al*., 2009), although area sensitivity of Grasshopper Sparrows may vary regionally (Johnson and Igl, 2001). Moreover, With *et al*. (2008) suggest that grassland bird declines observed today are the result of past habitat declines, and that the size of current patches may not be sufficient for their conservation. However, at least some birds may be able to exploit small grassland patches for breeding (Weidman and Litvaitis, 2011). Finally, grassland bird abundance and diversity may be influenced negatively by the presence of exotic grass species (Flanders *et al*., 2006; George *et al*., 2013; Ortega-S *et al*., 2013). These specific habitat requirements likely play a role in their decline, since grass cover is decreasing and shrub cover is increasing in many areas (Ceballos *et al.,* 2005; 2010).

Grassland management. Habitat characteristics are strongly influenced by habitat management practices (Klute and Robel, 1997; Bechtholdt and Stouffer, 2005; Desmond *et al*., 2005). For example, Desmond (2004) compared grassland bird abundance and diversity on communal lands (*ejidos*) where prairie dogs were present with private ranches with and without prairie dogs and found substantial differences between these two management types. *Ejidos* are a form of communal land ownership on which overgrazing is common. Private ranches are thought to use more rotational grazing practices leading to less grassland degradation. In this regard, Desmond (2004) found that winter bird diversity was higher on private ranches than on *ejidos*, with no difference between ranches with or without prairie dogs. Abundance was more influenced by the presence of prairie dogs, with the highest bird abundance on private ranches with prairie dogs, the second highest on *ejidos* with prairie dogs and the lowest bird abundance on private ranches without prairie dogs. Furthermore, incentive programs of the government can improve conservation management of private land owners (Klute *et al*., 1997; Seigel and Lockwood, 2010). Seigel and Lockwood (2010) showed that when half of the lands participated in these incentive programs, the extinction probability was lower than 10 %. Grassland birds may also be able to exploit perennial biofuel feedstocks (Robertson *et al.*, 2011). The most detrimental to grassland bird populations is conversion of grasslands to intensive agricultural areas (Pool *et al*., 2014). Birds cannot exploit these areas and furthermore, grassland bird abundance is limited along agricultural borders (Desmond *et al*., 2005). However, some birds seem able to breed in agricultural fields and overgrazed grasslands (Posadas-Leal *et al*., 2010), although this is not the case for Baird's and Grasshopper Sparrows (Desmond *et al*., 2005).

Diet and Seed Selection

Little information exists on the diet of Baird's Sparrow and Grasshopper Sparrow. However, some insight into which factors may be important in their diet can be learned from studies in similar species. Two diet-related factors that might play a role in grassland bird abundance and diversity are (1) food density within an area and (2) seed selection by different species.

Seed abundance. Several studies have shown an association between sparrow abundance and seed availability. For example, Grzybowski (1983) found a positive correlation between seed density and bird density in Texas and Oklahoma during winter. In this regard, Ginter and Desmond (2005) studied foraging behaviour of Savannah Sparrows (*Passerculus sandwhichensis*) in Texas and found seed biomass to be higher in areas where Savannah Sparrows foraged compared to randomly selected areas. Abundance of Henslow's

Sparrows (*Ammodramus henslowii*) was also correlated positively to seed density (Bechtholdt and Stouffer, 2005). Furthermore, sparrow abundance in winter has been positively correlated with preceding summer rainfall (Dunning and Brown, 1982; Macías-Duarte, 2009), which is a predictor of grass productivity (Khumalo and Holechek, 2005) and seed production (Pulliam and Parker, 1979).

Overall seed production, influenced by climate, also seems to be important in determining the relationship between seed abundance and bird abundance. Pulliam and Dunning (1987) found that sparrow abundance in arid woodland areas in Arizona was unrelated to seed biomass in years of moderate to high seed production. These authors therefore suggest that over-wintering sparrows are not food-limited every winter. However, Méndez-Gonzalez (2010) experimentally manipulated seed abundance in a year with low seed production and a year with high seed production and found that in both years avian abundance responded positively to the addition of seeds to a grassland area. Whether or not overall seed availability is important or not may therefore depend on other factors such as seed diversity and nutrient content of the available seeds.

Seed diversity. Desmond *et al*. (2008) compared different sites in New Mexico and found the highest seed biomass and diversity in an open grassland area dominated by annual grasses and forbs. This area also supported the highest bird abundance and diversity. Furthermore, they found high seed biomass but low seed diversity in an open mixed grassland area with both annual and perennial grasses, and this area supported a lower bird abundance and diversity. These results indicate that not just seed abundance but also seed diversity is important in determining grassland bird abundance and diversity. The findings are in agreement with Méndez-González (2010) who studied the effect of plant diversity, seed diversity, and subsequently avian diversity in relation to the abundance of exotic grass species. He compared winter bird community abundance and diversity in two different grassland sites in southeastern Arizona of which one was dominated by the exotic Lehmann lovegrass (*Eragrostis lehmanniana* Ness) and the other consisted of a mix of Lehmann lovegrass and native grass species. The findings showed a significantly higher plant diversity and avian abundance and diversity in the mixed site over two years of study. This indicates that avian diversity is positively related to seed diversity, and furthermore that the introduction of exotic plant species may have a negative effect on both.

Thus, several studies indicate that both seed abundance and diversity are related to overall grassland bird abundance and diversity in a particular area. However, these are general patterns and do not give information on a particular bird species. Whether or not a specific species will be able to survive in a particular habitat will depend, among other factors, on its diet, dietary plasticity and seed handling ability, which in turn influence seed selection. For each specific bird species, seed selection may be influenced by several characteristics of the seed such as seed size and morphology, seed abundance and visibility, toxicity, nutrient content and coat thickness (Díaz, 1994) and several characteristics of the bird, such as body size and bill morphology (Benkman and Pulliam, 1988; Méndez-Gonzalez, 2010).

Seed size. Seed selection could be influenced by the size of a seed in relation to body size and/or bill morphology of a bird, which results in differences in handling efficiency between birds for different seed sizes (Pulliam, 1985; Hrabar and Perrin, 2002; Van der Meij *et al.*, 2004). Optimal foraging theory states that animals select food items in order to maximize energy intake over time (Charnov, 1976). According to this theory, birds should select those seeds that they can handle most efficiently. Seed size is correlated with energy content (Willson, 1971). Therefore, optimal foraging may result in resource partitioning between bird species based on seed size. In this regard, many studies have found a relationship between bird or bill size and handling efficiency (Willson, 1971; Pulliam, 1983; Díaz, 1990; 1996; Soobramoney and Perrin, 2007). However, for optimal foraging theory to apply, larger billed birds should select larger seeds than smaller billed birds. In this regard, Pulliam (1983) showed a positive relationship between bill size of nine sparrow species and seed size of seeds eaten and Desmond *et al.* (2008) found that smaller-sized sparrows specialize on smaller seeds whereas larger sparrows prefer both smaller and larger sized seeds. However, others have found that both larger and smaller billed birds prefer smaller seeds instead of those that they handle most efficiently (Thompson *et al*., 1987; Keating *et al.*, 1992). Méndez-González (2010) did not find a relationship between bill size and seed size, and suggests that, among sparrows, resource partitioning is based on other factors than seed size. Benkman and Pulliam (1988) compared seed handling efficiency of sparrows and finches and found that the smaller sized sparrows were more efficient in handling small seeds weighing less than 0.74 mg, whereas the larger bodied finches were more efficient in handling larger seeds weighing more than 1.4 mg. Furthermore, in contrast to finches, they found no evidence of resource partitioning in sparrows, i.e., the three sparrows in their study - Chipping Sparrow (*Spizella passerina*), Dark-eyed Junco (*Junco hyemalis*) and White-crowned Sparrow (*Zonotrichia leucophrys*) - fed on the same seeds. They attribute this to the limited range of seed sizes used by sparrows (Benkman and Pulliam, 1988).

Thus, there is a large body of evidence suggesting that handling time influences seed selection. However, it is not clear whether this results in resource partitioning in the sense that smaller birds select smaller seeds and larger birds larger seeds, although larger billed birds appear to include a wider range of seed sizes in their diet (Willson, 1971; Desmond *et al*., 2008). Resource partitioning in sparrows may also depend on other factors than seed size such as resource availability. Especially when seeds are scarce high overlap of diet between different sparrow species can be expected, whereas more partitioning will occur when seeds are more abundant (Pulliam, 1985; 1986). Seed selection therefore seems not only based on seed size but also on other seed characteristics, and different bird species have been found to show different preferences.

Other seed characteristics. A comparison of the winter diet of five sparrow species in New Mexico (Chipping Sparrow, Brewer's Sparrow (*Spizella breweri*), Savannah Sparrow, Vesper Sparrow (*Pooecetes gramineus*), and White-crowned Sparrow) showed that the diet of some species contains up to 56 different seed species whereas the diet of other sparrows was limited to 15 different seed species (Méndez-Gonzalez, 2010). This indicates that some birds may be more selective than others. Seed selection may be related to energy and nutrient content, and the ability of a bird to handle a seed will in turn affect whether a bird is able to obtain its energy and nutrients. This can in turn be expected to differ between plant and bird species.

Desmond *et al.* (2008) showed considerable variation in seed selection and preference of five sparrow species in New Mexico, and related part of this variation to differences in body size. However, all birds preferred seeds from annual grasses and forbs with the exception of three perennial seeds (dropseed (*Sporobulus* spp.), panicum (*Panicum* spp.) and threeawn (*Aristada* spp.)). Dropseed was important in the diet of all five species and where available preferred by all but Savannah Sparrow. Of the smaller sized sparrows, Chipping Sparrows did not show any other preferences whereas Brewer's Sparrows preferred stinkgrass (*Eragrostis cilianensis*) next to dropseed. The larger bodied sparrows showed more variation and less specialization related to seed size. Next to dropseed, Vesper Sparrows preferred goosefoot (*Chenipodium* spp.) or knotweed (*Polygonum* spp.) and amaranth (*Amaranthus* spp.), depending on the habitat. Savannah Sparrows preferred cupgrass (*Eriochloa* spp.) and amaranth, and White-crowned Sparrows preferred amaranth next to dropseed. The preferred seeds were not always the most abundant seeds. For example vervain (*Verbena* spp.), clover (*Trifolium* spp.) and neckweed (*Veronica peregrine*) were abundant but not present in the diet of any of the five sparrow species. Preferred seeds were also not always the most common seeds in the diet. For example, feather fingergrass (*Chloris virgata*) was common in the diet of Chipping Sparrows and Savannah Sparrows. Finally, seed preferences changed over the season from mid- to late winter related to changes in seed abundance and changes in avian community composition. However, the diets of dominant species did not change as much as that of smaller birds (Chipping and Brewer's Sparrow) indicating that competition plays a role here (Desmond *et al*., 2008).

Thus, different sparrow species had different seed preferences, preferred seeds were consumed in higher amount in relation to their abundance, and some abundant seeds were never consumed. This suggest that seed preferences are important in seed selection and may therefore play a role in determining the suitability of a particular grassland area for a particular bird species, based on vegetation composition. It is not clear from this study where these seed preferences are based on, but the authors suggest that a combination of energy and nutrient content and seed size could be involved (Desmond *et al.*, 2008). Some evidence for the importance of energy content in seed selection is provided by DiMicelli *et al.* (2007) who studied seed selection in Henslow's Sparrows (*Ammodramus henslowii*) south-eastern U.S.A. in relation to fire management. Henslow's sparrow abundance in winter is higher in areas where burning took place the previous season and Dimicelli *et al*. (2007) showed that the driving factor explaining this between-year variation in distribution may be related to seed preferences of Henslow's Sparrows. They studied seed preferences in captive Henslow's Sparrows and found that these birds preferred seeds from grass species that are most common in the first winter after burning. Furthermore, they showed that these seeds had higher energy content than least preferred seeds. This therefore indicates that energy content may be one of the seed characteristic that is important in explaining distribution patterns of grassland birds.

Pulliam (1980) showed that seeds from forbs were the most common seeds in the diet of Chipping sparrows, whereas Cueto *et al*. (2006) found that granivorous birds in arid parts of Argentina select grass over forb seeds. The birds in the study of Cueto *et al.* (2006) showed a preference for larger-sized grass seeds but furthermore, they showed a preference for particular seed species over others despite similar seed size. This indicates that nutrient content or other seed characteristics indeed played a role in seed selection. Different studies have found different components to be of importance, such as energy content (Valera *et al*., 2005), fat (Thompson *et al*., 1987; Molukwu *et al*., 2011), protein (Valera *et al*., 2005; Larson *et al*., 2012; Johansen *et al*., 2014), carbohydrates (Molukwu *et al*., 2011; Ríos *et al*., 2012), and water content (Carillo *et al*., 2007). Birds may also avoid seeds with toxic components (Marone *et al*., 2008; Molukwu *et al*., 2011; Ríos *et al*., 2012). However, other studies have found no influence of nutrient components (Willson, 1971; Hrabar and Perrin, 2002; Soobramoney and Perrin, 2007), and it is suggested that chemical composition is of secondary importance compared to seed size (Díaz, 1996).

Molecular Seed Identification in Stomach Samples

Several studies used regurgitation to study the diet of grassland sparrows. Desmond *et al*. (2008) used this technique to study the diet of sparrow communities in different grassland habitats in New Mexico. Pulliam (1985) took regurgitated stomach samples to study the diet of Chipping Sparrows, and Marone *et al*. (2008) studied the diet of sparrow species in the Monte Desert of Argentina. These three studies identified the seeds in the stomach samples using a microscope and by comparing seeds with identified seeds from a reference collection and/or seed identification guides. However, this technique relies greatly on the expertise of the researcher and because many seed species are similar in morphology seed identification is based heavily on the subjective judgment of the investigator. Often it is not possible to identify seeds to species level. For example, Desmond *et al.* (2008) identified many seeds only to genus. Identification at the genus level is often insufficient as many grasslands may be dominated by species of the same genus (e.g. *Bouteloua*) and yet, size and energy content of their seeds vary significantly. The rapid development of molecular techniques allows for a more objective way to identify seeds in diet samples (Pompanon *et al*., 2012).

DNA barcoding. In DNA barcoding, a small, universal DNA sequence is used to identify samples. The DNA barcode is a sequence that is highly conserved but at the same time sufficiently variable to distinguish between species. The sequence is amplified by PCR using an established pair of universal primers, after which the PCR product is sequenced (Valentini *et al*., 2009a). The sequence can then be compared to a database such as GenBank (NCBI, 2014) or the Barcode of Life Data Systems (BOLD) for its identification (Cowan and Fay, 2012). Alternatively, a customized database can be designed by the researcher using sequences of species from a reference collection (Soininen *et al*., 2013). DNA barcoding can be combined with next-generation sequencing techniques in diet studies in which the diet samples contain numerous different species. These massive sequencing techniques assure that all sequences in the amplification product will be sequenced (Pompanon *et al.*, 2012). This technique has been used to study the diet of different species of herbivores including mammals, birds and invertebrates (Pegard *et al*., 2009; Valentini *et al*., 2009b; Soininen *et al.*, 2013). The method of DNA barcoding was first developed in animals in which the mitochondrial *CO1* gene serves as an established barcode (Hollingsworth *et al*., 2011). For plants it was more difficult to find one universal barcode. One of the most important characteristics to consider in the choice of a barcode is its ability to discriminate between species (i.e., its discrimination power). Different regions have been proposed as barcodes. The Consortium for the Barcode of Life (CBOL) officially recognized the combination of the regions *matK* and *rbcL* as a universal barcode for plants (Cowan and Fay, 2012). These two regions are coding regions in the chloroplast DNA. Whereas *matK* has a high discriminatory power, it can be difficult to amplify. On the other hand, *rbcL* has a lower discriminatory power but it is easier to amplify. The combined discrimination power of these two regions is around 70 %. An advantage of using coding regions is that the DNA sequences can be translated to amino acids which can be used to control for editing and/or assembly errors, pseudogenes and a correct sequence orientation (Hollingsworth *et al*., 2011). However, depending on the type of study and the species involved, different regions may be considered (Table 1).

Another commonly used barcode for plants is the intergenic spacer *trnH-psbA*. The advantages of this region are easy amplification, variability, and a high discrimination power in some species. The disadvantages of *trnH-psbA* are problems with the loci duplications, microinversions and a premature termination of the sequence reads in up to 30 % of the sequences (Hollingsworth *et al*., 2011). A shorter chloroplast region that has been used in the diet studies mentioned before is the P6 loop of the *trnL* intron. A major advantage of this region is the short sequence, for which the *trnL* intron is particularly valuable for identifying species in samples with degraded DNA. Other advantages are the high number of sequences in existing databases and the fact that the primers are extremely

Table 1. Summary of plant DNA barcode regions

DNA region	Genomic source	Amplicon length (bp)	Number of GenBank species	Species discrimination success (%)
matK	Plastid	862-910	22701	57-100
rbcL	Plastid	654	20374	90
trnH-psbA	Plastid	226-936	11539	25-73
trnL (P6 loop)	Plastid	51-135	38329	19-75
nrITS	Nuclear	407-1630	52450	23-97
nrITS2	Nuclear	157-670	57579	45-93

Adapted from Table 1 and Table S1 in Hollingsworth *et al*. (2011).

well conserved, which is important when multiple species are amplified in the same PCR. The disadvantage of this region is its low resolution in discriminating between species (Taberlet *et al*., 2007). For example, identification to species level was only 20 % (Taberlet *et al*., 2007) and 24.4 % (Pegard *et al*., 2009). However, Valentini *et al.* (2009b) report a discrimination success of 75 % to species level. Finally, two nuclear regions have been used as barcodes; nrITS and its shorter variant nrITS2. The advantage of nrITS is that it has a high discriminatory power. A disadvantage of this region is that there may be divergent paralogous copies within individuals, which can prevent a readable sequence from being obtained. Another disadvantage is that different variants of nrITS may be obtained depending on the amplification strategy, primers used and the PCR efficiency, which results in different species identification based on laboratory protocols and chance. The nrITS region may also have a problem with fungal contamination, and finally, it may be difficult to amplify and sequence. The shorter nrITS2 region is easier to amplify and sequence and is more length conserved, but has a reduced number of available characters in comparison to nrITS (Hollingsworth *et al*., 2011). Some characteristics of the discussed barcode regions are summarized in Table 1.

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STUDY 1. IMPORTANCE OF SEED CHARACTERISTICS IN DIET PREFERENCES OF GRANIVOROUS BIRDS: A PILOT STUDY WITH HOUSE SPARROWS (*Passer domesticus*)

RESUMEN

IMPORTANCIA DE LAS CARACTERISTICAS DE SEMILLAS EN LAS PREFERENCIAS POR AVES GRANIVORAS: ESTUDIO PILOTO CON EL GORRION INGLÉS (*Passer domesticus*)

POR:

M. Sc. MIEKE TITULAER

Doctor in Philosophia en Producción Animal

Secretaría de Investigación y Posgrado

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La disponibilidad de semillas que pueden ser aprovechadas forma un componente importante de la calidad del hábitat para las aves granívoras. Con el fin de obtener experiencia en estudios de selección de dieta en gorriones, se desarrolló este estudio piloto con el objetivo de investigar el efecto de diferentes características de las semillas (tamaño, color, visibilidad, composición de nutrientes) sobre la selección de semillas por el gorrión Inglés (*Passer domesticus*). Para lograr la adaptación a la dieta se ofreció una mezcla de nueve semillas comerciales y después se corrieron seis pruebas con diferentes combinaciones de tres semillas que variaron en solo una de las características de interés. De las características bajo estudio, solo el tamaño influyó la selección de semillas. Los gorriones prefirieron semillas de tamaños intermedios en todas las pruebas. Con base en estos resultados, se plantea la hipótesis de que los gorriones seleccionan semillas que pueden manipular más eficientemente para

maximizar el consumo de energía sobre el tiempo. Por lo tanto, es importante incluir características de la cubierta y el tiempo de manipulación en estudios futuros sobre la selección de semillas.

ABSTRACT

IMPORTANCE OF SEED CHARACTERISTICS IN DIET PREFERENCES OF GRANIVOROUS BIRDS: A PILOT STUDY WITH HOUSE SPARROWS (*Passer domesticus*)

BY:

MIEKE TITULAER

The availability of seeds that can be consumed profitably is an important aspect of habitat quality for granivorous birds. In order to gain experience with diet selection studies in sparrows, a pilot study was performed with the objective of investigating the effect of different seed characteristics (size, color, visibility, nutrient composition) on seed selection by House Sparrows (*Passer domesticus*). For adaptation to the diet, a mixture of nine commercial seed types were offered and subsequently, six trials with different combinations of three seed types that varied on one of the three characteristics of interest were ran. Of the characteristics under study, seed size was the only one influencing seed choice. House Sparrows preferred seeds of intermediate size in all trials. Based on these results, it is hypothesized that House Sparrows may be selecting seeds that they can handle most efficiently to maximize energy intake over time. Therefore, it is important to take husk characteristics and handling time into account in future studies on seed selection.

INTRODUCTION

Habitat suitability is a key indicator in wildlife management. The design of appropriate management plans to protect declining granivorous bird species requires insight into the factors that influence habitat suitability for these birds. Optimal foraging theory predicts that birds should select seeds to maximize the rate of energy intake with respect to searching and handling time (Krebs *et al*., 1978). Accordingly, food availability and distribution, as well as the characteristics of the available seeds in an area, are important factors influencing habitat suitability. For example, seed handling efficiency influences which seeds can be consumed profitably by different bird species and therefore, ultimately, which habitats are adequate for their survival (Benkman and Pulliam, 1988). In this regard, different bird species have been found to specialize on different seed types in the field (Desmond *et al*., 2008). Seed selection may furthermore be influenced by different characteristics of the seed such as size, color, structure, nutrient content, visibility and toxicity (Díaz, 1994). Research on seed selection by granivorous birds has mainly focused on the effects of seed size in relation to bill and/or body size (Willson, 1971; Pulliam, 1983; Keating, 1992; Soobramoney and Perrin, 2007). Furthermore, several bird species have been found to select their food items based on energy content (DiMicelli *et al*., 2007) or some essential nutritional component (Carillo *et al.*, 2007; Senar *et al*., 2010; Molukwu *et al*., 2011; Ríos *et al*., 2012; Johansen *et al*., 2014). Fruit eating birds have been shown to express color preferences when certain colors are related to essential nutrients (Schmidt and Schaefer 2004; Schaefer *et al*., 2008). In this regard, granivorous birds face the challenge of not only foraging on sparsed seeds but also discriminating between the quality of food items based on characteristics that are not as evident as those for insectivorous or frugivorous birds.

This study investigated seed preferences by House Sparrows (*Passer domesticus*) in a pilot study with two objectives: (1) to try different procedures that could potentially be used in seed choice experiments with wild granivorous birds, and (2) to investigate the importance of the seed characteristics size, color and visibility in seed selection.

MATERIALS AND METHODS

The pilot study took place in November 2012. Five adult male house sparrows were captured with traps and a bird attractor around Chihuahua City, Mexico. Immediately after capture, birds were weighed and bill length, width, and depth were measured to the nearest 0.1 mm with a caliper. At the end of the test, birds were released in the area of capture. Birds were housed in cages of 0.8 × 0.8 × 0.8 m with a swing, perch, nest and *ad libitum* access to water. The diet consisted of a mixture of nine commercial seeds that were used in the seed selection experiments: canary grass (*Phalaris canariensis*), niger (*Guizotia abyssinica*), yellow and red millet (*Panicum miliaceum*), rapeseed (*Brassica napus*), wheat (*Triticum sativum*), sorghum (*Sorghum bicolor*), amaranth (*Amaranthus hypochondriacus*), and sunflower (*Helianthus annus*) seeds. These seeds were chosen because of their variation in size and color. Nutrient content (moisture, ash, protein and fat) of the nine seeds was determined with a bromatological analysis (AOAC, 1990). Length (L), width (W) and depth (D) of 10 seeds of each type was determined to calculate a seed volume index (L*W*D) per seed type. Additionally, the weight of 10 seeds of each type was determined to calculate the mean seed weight per seed type. Information on seed size and nutrient analysis are shown in Table 2.

On experimental days, all food was removed from the cage at 1700. The trials started at 0700 the next day, with only one trial per day. After the experimental trial, birds were fed a mixture of the nine seed types until 1700. Feeding trays contained three equal compartments. The experiment was initiated one week after birds were captured and consisted of two phases. During Phase 1

Table 2. Water and nutrient content, and size of the nine experimental seed types

Seed Type	Moisture (%)	Ash (%)	Ether Extraction Fat (%)	Crude Protein (%)	Weight (mg)	Volume (mm^3)
Amaranth	2.15	3.08	7.62	18.32	0.71	4.32
Canola	2.47	3.67	38.75	22.48	3.46	5.41
Niger	3.23	3.98	34.87	22.86	4.02	6.44
Red Millet	4.97	2.79	4.23	14.40	4.69	10.49
Yellow Millet	6.06	2.66	4.19	13.25	6.28	13.42
Canary Grass	5.67	8.68	7.05	15.92	7.41	15.40
Sorghum	5.48	0.74	3.30	10.62	31.62	45.54
Wheat	4.00	1.66	2.72	16.02	33.44	46.62
Sunflower	2.17	2.77	38.73	18.18	55.21	165.57

(Days 1-2), the birds were presented with a mixture of 1 g of each seed type equally divided over the three compartments of the feeder. On Day 1, feeding time was 30 min and on Day 2, 6 h. At the end of the feeding time, the remaining seeds were removed and the amount consumed of each seed type was determined as the difference in mass between the end and the beginning of the feeding period. Seed preferences were determined by comparing the amount consumed of each seed type. In Phase 2 (Days 3-8), birds were offered a combination of three seeds that varied in only one of three characteristics of interest: size, color or visibility. To test the effect of size, birds were offered three seeds of the same color but different sizes. For the color effect trials, canary grass seeds were painted with an artificial colorant without odor or flavor. For the visibility effect trials, birds were presented with two seed species with the same color as the feeder (no contrast, less visible) and one seed species with a different color than the feeder (high contrast, more visible). The expectation was that, if visibility would play a role in seed selection, birds would prefer the most contrasting seed. In total, we ran six trials, two for each seed characteristic. The order of the trials and the compartment in which each of the three seed types was placed were determined at random. Feeding time in Phase 2 was 45 min, because on day 1 of Phase 1 birds consumed very little in 30 min.

Data of Phase 2 were analyzed with a linear mixed model with the logarithm of the amount of seeds consumed (g) as response. Normality of the log-transformed variable was confirmed using a Q-Q plot. Seed type, trial, and their interaction were fitted as fixed effects, so the model becomes

$$\log(\textit{seed consumption}) = \textit{SEED TYPE} + \textit{TRIAL} + \textit{SEED TYPE} \times \textit{TRIAL}$$

The main interest was in the interaction, because a significant interaction would imply that in at least one of the six trials, one of the three seeds was consumed in a different amount than the other two. In other words, it would mean that at least one of the three seeds in at least one of the six trials was preferred or avoided. Bird weight (g) and bill volume (L*W*D) were added as covariates. To control for pseudo-replication, individual (bird) was included in the model as a random effect. The final model was selected through the backward elimination of non-significant terms. Analyses were run in R 2.13.1. Pairwise comparisons were performed to investigate statistical differences among the three seed types in each of the six trials with a z-test using the glht function and specified contrasts (Table 3).

Table 3. Specified contrasts to compare the amount consumed of seeds within each trial in Phase 2

Trial	Characteristic	Contrast 1	Contrast 2	Contrast 3
1 - 2	Size	Small *vs.* intermediate	Small *vs*. large	Intermediate *vs.* large
3 - 4	Color	Yellow *vs*. red	Yellow *vs*. black	Red *vs*. black
5 - 6	Visibility	More visible *vs*. less visible 1	More visible *vs*. less visible 2	Less visible 1 *vs*. less visible 2

RESULTS AND DISCUSSION

Results of Phase 1 showed that birds preferred canary grass (Figure 1). This was the only seed consumed when feeding time was only 30 min (Day 1). With a longer feeding time (Day 2), birds consumed mostly millet after canary grass was totally consumed, but one bird preferred niger. Preferences did not seem to be related to fat or protein content (Table 2). What distinguished the preferred seed types from the others was mainly its size. Volume (mm^3) of canary grass and millet is intermediate among the seed types in this experiment. Another characteristic of canary grass seeds is that the husk is less hard. Birds remove the husk from the seed before consuming it. Thus, handling time may have been shorter for canary grass.

In Phase 2, there was a significant interaction between seed type and trial ($F_{10,68} = 2.95$, $P = 0.004$), indicating that seed type had a significant influence on the amount consumed in at least one of the six trials. The specified contrasts (Table 3) indicated that in the first trial for the characteristic seed size, birds preferred seeds of intermediate size over the smaller ($P = 0.002$) and larger ($P = 0.066$) seeds (Figure 2A). The same pattern for a size effect was observed in the second seed size trial (Figure 2B), but these differences were not significant ($P = 0.37$ and $P = 0.30$, respectively). Canary grass was never used in the seed size trials because Phase 1 showed that canary grass was the preferred seed. To prove that intermediate seed size (among the sizes of the seed types offered) was indeed a preferred seed characteristic, it was decided to use other seeds of intermediate sizes than the most preferred one. In contrast, canary grass was used in the color and contrast trials because, if these characteristics were

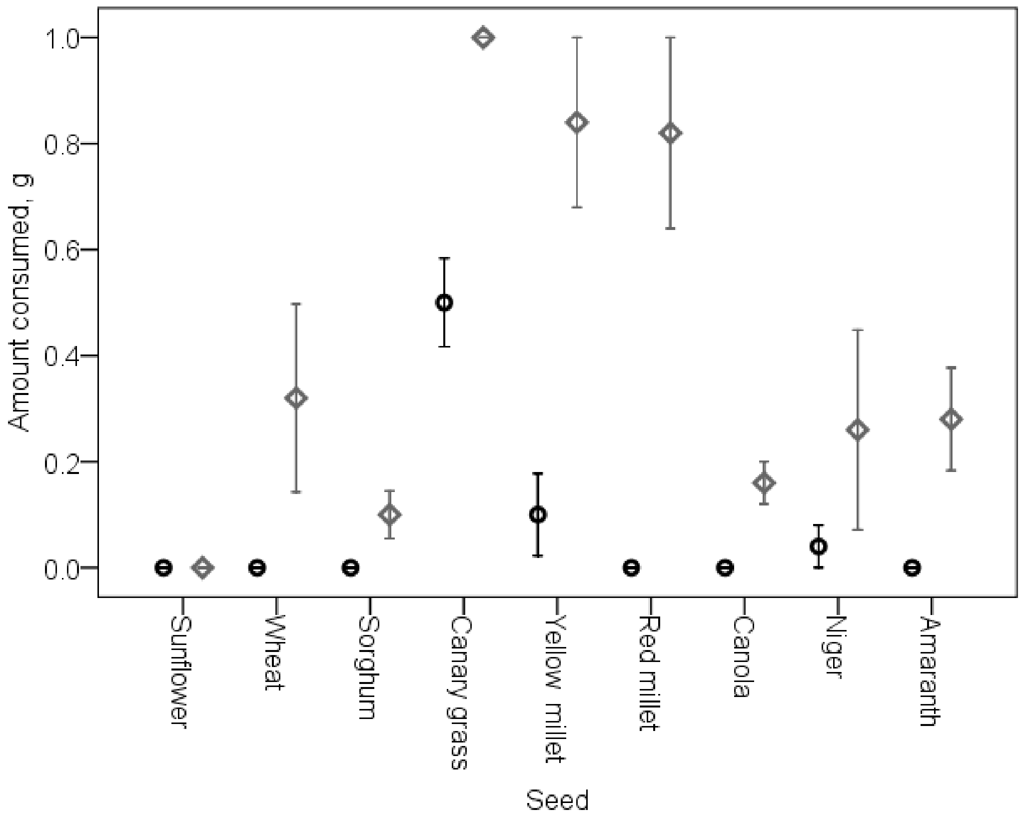

Figure 1. Mean amount consumed (± S.E.) of each seed type after a feeding time of 30 min (black circles) or 6 h (gray diamonds) in *Passer domesticus*.

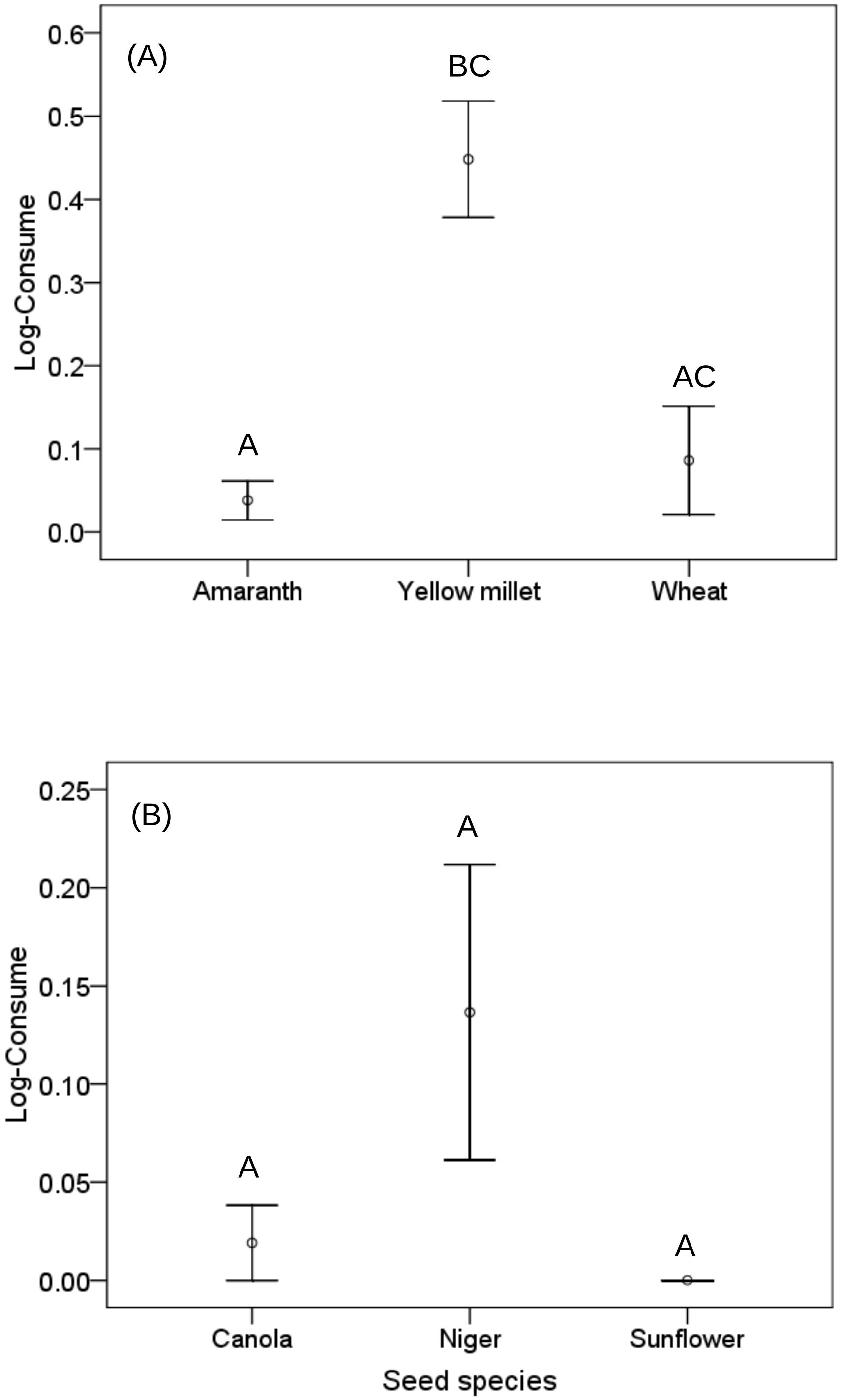

Figure 2. Mean amount consumed (± S.E.) (Ln transformed) of the three seed species in trial 1 (A) and trial 2 (B), testing for the effect of seed size on preferences of *Passer domesticus*. Different letters above the error-bars indicate statistical difference (P<0.05) based on the specified contrasts. Seeds are ordered by size from the small to large.

important, birds should choose the preferred color and most contrasting seeds instead of canary grass. However, there was no relationship between color or contrast and seed preference in any of the trials testing for these characterisitics (all $P > 0.05$). Rather than preferring seeds of a specific color or seeds that contrasted most with the feeder, birds consistently preferred canary grass or millet, which is consistent with seed preferences found in phase 1 of the study. Neither bill volume nor body weight ($P > 0.05$) influenced seed selection. However, there was little variation in body weight (mean = 24.3 g ± 1.0) among trial subjects. Altough there was some variation in bill volume (mean = 798.7 mm^3 ± 107.4) this may still be limited compared to inter-species variability. Therefore, body weight and bill volume may be more useful to measure when comparing different bird species.

In summary, the results indicate that the only important characteristic influencing seed preferences of House Sparrows is seed size. This finding is in agreement with previous research (Willson, 1971; Pulliam, 1983; 1985; Benkman and Pulliam, 1988; Keating, 1992; Díaz, 1996; Hrabar and Perrin, 2002). There was no effect of seed color or visibility on seed preference. This is in contrast to the color effect observed in fruit eating birds, where a red color is related to ripeness (Schmidt and Schaefer, 2004). Granivorous birds may not show color preferences because seed color is not consistently related to any desired nutrient content. Alternatively, granivorous birds may not show color preferences because seeds are usually buried in the soil. House sparrows have relatively large bills. Preferences for intermediate seed sizes may indicate that birds are selecting the largest seeds that they can still handle efficiently, as to maximize energy intake over time (Pulliam, 1985; Benkman and Pulliam, 1988). In this regard, seed size rather than nutrient content seemed to influence preferences in Phase 1 of this study. Previous studies also show that seed size is more important than nutrient content (Díaz, 1996). However, husk characteristics may also play a role in handling efficiency and should be distinguished from seed size (Van der Meij *et al.,* 2004). Thus, this pilot study shows the importance of measuring handling time in future studies, and to take into account husk characteristics in addition to seed size. Finally, it should be emphasized that this was a pilot study and that a larger sample size is required in subsequent experiments because of substantial individual variation.

CONCLUSIONS AND RECOMMENDATIONS

The results of this pilot study indicate that seed size may be one of the most important seed characteristics involved in seed selection. Seed color, visibility and nutrient content did not appear to influence seed selection by House Sparrows. The importance of seed size has previously been related to handling effiency, which is in turn determined by bill size (Díaz, 1996). However, seed size is not the only factor influencing handling efficiency. Other husk characteristics such as hardness and the presence of awns may also play a role (Van der Meij *et al*., 2004). Therefore, based on this pilot study, it is recommended that studies on seed selection should measure handling efficiency and take into account seed size as well as husk characteristics.

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STUDY 2. CAN GRASSLAND BIRDS EAT NON-NATIVE GRASS SEEDS? SEED PREFERENCES OF THREE DECLINING SPARROWS

BY:

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RESUMEN

PUEDEN LAS AVES DE PASTIZAL COMER SEMILLAS INTRODUCIDAS? PREFERENCIAS DE SEMILLAS EN TRES GORRIONES EN DISMINUCIÓN

POR:

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La invasión de zacates exóticos puede ser una amenaza para el hábitat invernal de las aves granívoras de pastizal debido a la reducción de semillas nativas. El objetivo fue comparar las preferencias de semillas de *Ammodramus bairdii*, *A. savannarum* y *Passerculus sandwichensis* entre tres especies de zacates nativos: navajita (*Bouteloua gracilis*), banderita (*B. curtipendula*) y gigante (*Leptochloa dubia*); y tres especies invasoras: africano (*Eragrostis lehmanniana*), buffel (*Pennisetum ciliare*) y rosado (*Melinis repens*). Se sometió a prueba la hipótesis que la variación en las preferencias de semillas está relacionada con las diferencias en el tamaño del pico y la eficiencia de manipulación. Se ofrecieron semillas en pruebas de opción múltiple (todas las semillas al mismo tiempo) y de opción simple (una semilla a la vez). Los resultados muestran diferencias en preferencias de acuerdo con la hipótesis planteada. Cuando las aves no tenían opción, fueron capaces de aceptar todas las semillas con excepción de africano (la semilla más pequeña) y buffel (la

semilla más grande). Los resultados sugieren que ciertos zacates invasores podrían ser una fuente de alimento para aves invernales de pastizal; también muestran la importancia de limitar las plantaciones de zacate buffel y zacate africano y controlar sus invasiones, con el fin de conservar los recursos alimenticios de aves de pastizal.

ABSTRACT

CAN GRASSLAND BIRDS EAT NON-NATIVE GRASS SEEDS? SEED PREFERENCES OF THREE DECLINING SPARROWS

BY:

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The invasion of exotic grass species is a potential threat to the winter habitat of Northamerican migratory grassland birds by reducing native sources of seed food. The objective of this study was to compare seed preferences of Baird's Sparrows (*Ammodramus bairdii*), Grasshopper Sparrows (*A. savannarum*) and Savannah Sparrows (*Passerculus sandwichensis*) between three native grass species: blue grama (*Bouteloua gracilis*), sideoats grama (*B. curtipendula*), and green sprangletop (*Leptochloa dubia*); and three invasive species: Lehmann lovegrass (*Eragrostis lehmanniana*), buffelgrass (*Pennisetum ciliare*), and natal grass (*Melinis repens*). We hypothesized that variation in preferences would be related to differences in bill size and therefore handling efficiency rather than their origin (native *vs*. exotic). We offered seeds to captive birds in choice trials (all seeds at the same time) and non-choice trials (one seed per trial). The results show preference differences between the bird species in line with expectations based on bill size and handling efficiency. Handling time increased with seed size, but more so for smaller billed birds. In the choice trials, Baird's and Savannah Sparrows preferred natal grass, an intermediate sized seed with a short handling time. Grasshopper Sparrows, with the largest bills, preferred sideoats grama, a large seed. When birds had no choice, they were able to accept all seeds except for Lehmann lovegrass (the smallest seed) and buffelgrass (the largest seed). Our

findings suggest that certain invasive grasses may be a source of food for wintering grassland birds but also highlight the importance of limiting plantings of buffelgrass and Lehmann lovegrass and controlling their invasions in order to conserve food resources in grassland bird habitats.

INTRODUCTION

North American grassland birds are among the most threatened groups of birds worldwide (Brennan and Kuvlesky, 2012) as a result of long-term habitat decline and alterations in their breeding range (Askins *et al*., 2007; With *et al*., 2008). Most species of grassland birds are migratory and overwinter in the grasslands of northern Mexico (Manzano-Fischer *et al*., 2006). Reduction in suitable winter habitat is probably a key factor in grassland bird declines (Vickery and Herkert, 2001; Pool *et al*., 2014). Main threats to grassland bird winter habitat include large-scale agricultural conversions (Pool *et al*., 2014), overgrazing (Curtin *et al*., 2002; Desmond *et al*., 2005), shrub encroachment (Desmond *et al*., 2005) and invasion of exotic grass species (Van Devender *et al*., 2005; Ortega-S *et al*., 2013).

Limited attention has been focused on the effects of exotic grass invasions on wintering grassland bird populations. However, changes in vegetation characteristics due to invasive grass species have been shown to negatively influence breeding grassland birds (Flanders *et al*., 2006; George *et al*., 2013). Exotic grass invasions change structural characteristics of the vegetation and may reduce plant species richness and diversity (Steidl *et al*., 2013), which could lead to a reduction in food availability for seed-eating birds. The most widespread invasive grass species in the Chihuahuan Desert grasslands are natal grass (*Melinis repens* (Willd.) Zizka), Lehmann lovegrass (*Eragrostis lehmanniana* Nees) and buffelgrass (*Pennisetum ciliare* (L.) Link) (Ortega-S *et al*., 2013). Natal grass and Lehmann lovegrass are aggressive competitors of native grasses and already dominate large areas of northern Mexican grasslands and continue to spread (Melgoza-Castillo and Morales-Nieto, 2013; Melgoza-Castillo *et al*., 2014). Buffelgrass has been planted extensively in northern Mexico and southern U.S.A. to improve forage production of rangelands (Franklin *et al*., 2006) and remains popular among landowners due to its high forage quality and favorable characteristics such as drought tolerance (Brenner, 2010). However, buffelgrass may have similar negative effects on native vegetation characteristics (Sands *et al*., 2009) and bird communities (Flanders *et al*., 2006). The precise extent to which exotic grasses have invaded the Chihuahuan Desert has not yet been quantified. However, in monitored transects Lehmann lovegrass expanded cover for 200 % over a 20 year period, displacing 80 % of native forbs and 50 % of native grasses (Sánchez-Muñoz, 2009). Natal grass invasions are more recent but it is estimated to represent 5-10 % of the vegetation cover in the state of Chihuahua (Melgoza-Castillo *et al*., 2014).

Wintering grassland passerines, especially Emberizids, feed almost exclusively on seeds (Desmond *et al*., 2008). Therefore, habitat quality for these seed-eating birds may be strongly influenced by the presence of seeds that can be profitably exploited (Pulliam, 1986). Intriguingly, granivorous passerines have been shown to be selective in their diet in that they do not consume seeds relative to their abundance (Pulliam *et al*., 1985; Desmond *et al*., 2008). Seed selection may be influenced by handling time, which is a result of the relationship between seed size or form and bill characteristics (Willson, 1971; Díaz, 1996; Hrabar and Perrin, 2002; Van der Meij *et al*., 2004). Additionally, seed selection may be influenced by seed nutritional value such as energy (Valera *et al*., 2005), protein (Johansen *et al*., 2014), fat (Thompson *et al*., 1987; Molukwu *et al*., 2011), carbohydrate (Ríos *et al*., 2012), water content (Carillo *et al.*, 2007) or even the presence of toxic components (Molukwu *et al.*, 2011; Ríos *et al*., 2012).

Since invasive grass species tend to become dominant and reduce plant species richness and diversity (Sands *et al.,* 2009; Lyons *et al*., 2013), invasive grass species are a potential threat to granivorous grassland birds if birds are unable to exploit their seeds. In this regard, grassland bird abundance and species diversity was reduced at a winter habitat site in Arizona dominated by Lehmann lovegrass compared to a site where native grasses and Lehmann lovegrass were mixed (Méndez-González, 2010). However, more detailed information on seed selection by wintering grassland birds in relation to invasive grasses is lacking.

Baird's (*Ammodramus bairdii*), Grasshopper (*A. savannarum*) and Savannah Sparrows (*Passerculus sandwichensis*) are relevant species to study the effect of invasive grass species on grassland bird conservation. Baird's and Grasshopper Sparrows are grassland obligates (Vickery *et al*., 1999) that overwinter in northern Mexico (Panjabi *et al*., 2010). Savannah Sparrow has been listed as grassland obligate (Vickery *et al*., 1999) as well as grassland associate (Desmond *et al*., 2005) and often co-occurs with *Ammodramus* species during winter (Grzybowsky, 1983; Gordon, 2000). Widespread population declines have been documented for these three species over the last 40 years (Sauer *et al*., 2011). Because Baird's, Grasshopper and Savannah Sparrows overwinter in (semi-) open grasslands with tall, dense grass cover (Macías-Duarte *et al*., 2009), these species may be particularly vulnerable to grassland invasions by exotic species. These three sparrow species have similar body sizes but differ in bill morphology, which allows us to explore the role of bill morphology in seed selection controlling for energetic needs (i.e., metabolic rate).

Here, we investigated seed selection by Baird's, Grasshopper and Savannah Sparrows comparing consumption of seeds from the three main invasive grass species in northern Mexico (natal grass, Lehmann lovegrass and buffelgrass) to consumption of seeds from three native grasses. We predicted that (1) the ability of birds to exploit the invasive grass species would depend on their ability to handle these seed efficiently, and (2) that the three bird species would show differences in preferences and the ability to exploit different seeds as a result of bill size variability.

MATERIALS AND METHODS

Subjects and Housing

We used three sparrow species of similar body size but different bill morphology to explore the relationship between bill size and the ability to exploit different seeds: Baird's Sparrow (8 individuals), Grasshopper Sparrow (7 individuals) and Savannah Sparrow (7 individuals). We captured birds on 15-16 November 2013, at the research ranch Teseachi of the Autonomous University of Chihuahua, located in the municipality of Namiquipa, Chihuahua, Mexico (28º32'38" N, 107º26'45" W). We housed birds in indoor facilities inside the ranch, in individual cages of 1 × 0.5 × 0.5 m with four perches, a nest, and dried bean plant and grass (without seeds) as substrate. Vitamin enriched water was provided *ad libitum*. Diet before the experiment consisted of a mixture of the experimental seeds supplemented with commercial seeds (canary grass and millet). We measured bill length, width, and depth to the nearest 0.1 mm with a caliper and body mass to the nearest 0.1 g.

Experimental Seeds

We used blue grama (*Bouteloua gracilis* (Kunth) Lag. ex. Griffiths), sideoats grama (*B. curtipendula* (Michx.) Torr.), and green sprangletop ((*Leptochloa dubia* (Kunth) Nees) as native seed species (Figure 3). Blue grama and sideoats grama have been associated with Baird's, Grasshopper and Savannah Sparrow (Desmond *et al*., 2005). Green sprangletop is of intermediate size and also present in the habitat of the birds, although less common. We used natal grass, Lehmann lovegrass, and buffelgrass as exotic seed species (Figure 3), because these species are the most widespread invasive species in northern

Figure 3. Experimental seeds in order of seed mass: Lehmann lovegrass (*Eragrostis lehmanniana*), blue grama (*Bouteloua gracilis*), green sprangletop (*Leptochloa dubia*), natal grass (*Melinis repens*), sideoats grama (*Bouteloua curtipendula*) and buffelgrass (*Pennisetum ciliare*).

Mexico (see introduction). We collected all seeds throughout the state of Chihuahua in September and October 2013. Seed mass is correlated with seed energy content (Willson, 1971). We determined seed mass until the nearest 0.01 mg for 50 seeds of each species to calculate average seed mass per species. Blue grama, natal grass and green sprangletop were weighed in groups of five seeds and Lehmann lovegrass seeds were weighed in groups of 10. We offered unhusked seeds to the birds without removal of any seed structures as a representation of how birds will encounter the seeds in the field (Figure 3). For both native and exotic seed species, we used one small, one intermediate and one large seed species, therefore seed size and origin (i.e., native *vs*. exotic) were not related.

Experimental Procedure

The experiment took place from 19 to 27 November 2013, after a 3 to 4 day adaptation period during which birds were allowed to familiarize themselves with their captive situation and the experimental seeds. We followed this procedure because the introduced seed species are not present at the ranch, and birds might initially avoid them due to unfamiliarity. We provided commercial seeds only after a bird had sampled all the different experimental seeds. The evening before experimental days, we removed all seeds from the cages at sunset (approximately 1730 h) and covered cages with blankets. Cages were equipped with trays that captured all spilled seeds. We removed all seeds from the trays to leave none available to the birds. At sunrise the next morning (approximately 0700 h), we placed the experimental seeds in the cages. Birds were allowed 1 h to feed after which we removed the experimental seeds, including spilled seeds from the trays. We then fed the birds with a mixture of the experimental seeds supplemented with commercial seeds until sunset after which the same procedure was followed.

Following recommendations by Cueto *et al.* (2001), we tested preferences in choice trials and nonchoice trials. In choice trials, from day 1 to 3, we presented the six seed species simultaneously to the birds in individual feeders in random order. During nonchoice trials, from day 4 to 9, only one of the six seed species was presented at a time. The duration of the feeding trials was 1 h. Feeders were 10 cm high with a diameter of 15 cm, which allowed birds to enter inside the feeder to minimize spilling of seeds. In the case that seeds were spilled, they were recovered from the trays after each trial. We weighed seeds to the nearest 0.0001 g before and after the trial to determine the amount consumed. In choice trials we provided 2 g and in non-choice trials 4 g of each seed. These amounts were much higher than what the birds consumed to avoid that a preferred seed species might be depleted which would force birds to consume a less preferred species. We filmed one bird of each species every day during both the adaptation and experimental phase. We used these recordings to determine the handling times based on 40 seeds per grass and bird species. However, for Savannah sparrows handling times could not be obtained for buffelgrass and Lehmann lovegrass seeds because these seeds were never consumed on recordings.

Statistical Analysis

To verify the assumption that the bird species differ in bill morphology but not in body size we analyzed differences in bill volume (l × w × h) and body mass (as an indicator of body size) using univariate one-way Analysis of Variance (ANOVA) with bird species as factor and bill volume or body mass as response variables. To test the assumption that differences in bill morphology lead to differences in handling time, we analyzed differences in handling time (log-transformed) using a full-factorial ANOVA with bird species and seed species as fixed factors. The model assumption of normal distribution of error was checked graphically and using a Shapiro-Wilk normality test on the residuals. Analyses were performed in package *stats* of program R 3.1.1 (R Development Core Team, 2014).

Consumption of the different seeds in the choice trials is a multivariate response (Roa, 1992). Therefore, we analyzed the choice trials with a Dirichlet regression using the DirichletReg package (Maier, 2014) in R 3.1.1 (R Development Core Team, 2014). A Dirichlet regression is a statistical procedure to analyze compositional data and does not rely on the assumptions of homogeneity of variances among levels or a normal distribution (Maier, 2014). The response variable consumption was transformed to a compositional variable in which the proportions of each seed consumed added up to one for each individual bird. We performed model comparisons using a likelihood-ratio test for the deviance between models with linear predictors *BIRD SPECIES*, and *BIRD SPECIES* + *day* and the intercept-only model.

The data of the nonchoice trials can be analyzed using univariate analysis since the consumption of the different seed species is independent when the different seeds are not offered simultaneously (Roa, 1992). We fitted a linear mixed model to the data with bird species, seed species and their interactions as explanatory fixed factors, and consumption per seed species as the response variable. Individual was added as a random factor to control for pseudo-replication, and day was added as a random factor to control for variability between trials on different days. Model assumption of normality of errors was checked graphically and using a Shapiro-Wilk normality test on the residuals. Model comparisons were performed to decide which variables to retain in the final model based on a likelihood-ratio test for the deviance between the full model and simpler models. The analysis was performed in R 3.1.1 (R Development Core Team, 2014) using the lme4 package (Bates *et al.,* 2014). Pairwise comparisons of specified contrasts were performed using the glht function of the multcomp package (Hothorn *et al*., 2008) to determine the nature of the significant interaction between bird and seed species.

Ethical Note

The experiments were approved by SEMARNAT (the Mexican ministry of environment and natural resources, permit number SGPA/DGVS/09559/13 granted to AMD). Cages were adjusted as much as possible to the needs of the birds (see subjects and housing). We measured fat score on a scale from 0 (no fat) to 5 (bulging) as an indication of body condition. At the end of the experiment, we repeated the measurement of weight and fat score to compare body condition before and after the experiment. Fifteen of the 22 birds increased their body weight and fat score during their time in captivity, four birds remained the same and three birds lost weight. There was no pattern among species. On 27 November we released the birds in the same location as where they were caught.

RESULTS AND DISCUSSION

Bird species differed significantly in bill volume (one-way ANOVA, $F_{2,21}$ = 49.69, P < 0.001) but not in body mass (one-way ANOVA, $F_{2,21}$ = 1.53, P = 0.240), the latter an indicator of body size. Grasshopper Sparrow had the largest bill volume followed by Baird's Sparrow and finally Savannah Sparrow (Table 4). We found a significant interaction between bird and seed species for the handling time data (factorial ANOVA, $F_{8,606}$ = 3.23, P = 0.001), indicating that handling times differed between bird species for at least one of the seed species. Overall, handling time increased with an increase in seed size for all birds, but more so for Savannah Sparrow, the bird with the smallest bill volume (Table 5). Natal grass was an exception with a shorter handling time than some smaller seeds (Table 5).

Composition of consumed seeds in the choice trials evidently differed among species (Figure 4). Savannah Sparrows had a clear preference for natal grass seeds over the other seeds, with a second preference for blue grama, although consumption of this seed was only 30 % of that of natal grass. Baird's Sparrows also preferred natal grass seeds with secondary preferences for blue grama and sideoats grama, whereas Grasshopper Sparrows preferred sideoats grama with a second preference for natal grass (Table 5). The model including bird species performed significantly better than the intercept-only model (Likelihood-ratio test, D = 91.37, df 12, P < 0.001), indicating that bird species is an important variable in explaining variation in seed selection. Furthermore, the model including explanatory variables day and species was significantly better than the model only including factor species (Likelihood-ratio test, D = 39.12, df

Table 4. Bill measurements of Grasshopper (GRSP), Baird's (BAIS) and Savannah (SAVS) sparrows[1] (mean ± S.D.)

Bird species	Bill length[2] (mm)	Bill width[2] (mm)	Bill depth[2] (mm)	Bill volume[2] (mm^3)
GRSP	10.57 (0.43)^a	5.81 (0.35)^a	5.99 (0.32)^a	368.08 (35.87)^a
BAIS	10.15 (0.26)^b	5.78 (0.17)^a	5.59 (0.12)^b	327.59 (16.06)^b
SAVS	9.97 (0.23)^b	5.10 (0.16)^b	4.96 (0.31)^c	252.18 (20.30)^c

[1] *Ammodramus savannarum*, *A. bairdii* and *Passerculus sandwichensis*, respectively

[2] Letters indicate significant differences at P < 0.05 (Tukey test).

Table 5. Seed mass (mean ± S.D.), handling times (mean ± S.D.), and preference ranks based on the amount consumed in the choice trials for Grasshopper (GRSP), Baird's (BAIS) and Savannah sparrow (SAVS)[1]

Seed	Mass (mg)	Handling time (s) and preference rank		
		BAIS	GRSP	SAVS
Lehmann lovegrass	0.12 (0.03)	0.59 (0.29) - 6	0.59 (0.28) - 5	—[2] - 3
Blue grama	0.32 (0.06)	0.64 (0.38) - 3	0.47 (0.29) - 2	0.74 (0.34) - 2
Green sprangletop	0.60 (0.08)	0.72 (0.39) - 4	0.74 (0.31) - 4	0.79 (0.38) - 4
Natal grass	0.65 (0.11)	0.43 (0.32) - 2	0.41 (0.28) - 1	0.45 (0.33) - 1
Sideoats grama	2.44 (0.80)	0.89 (0.69) - 1	0.66 (0.81) - 3	1.51 (1.23) - 6
Buffel grass	2.88 (0.90)	1.15 (1.08) - 5	1.25 (1.01) - 6	—[2] - 5

[1] *Ammodramus savannarum*, *A. bairdii* and *Passerculus sandwichensis*, respectively

[2] SAVS never consumed Lehmann lovegrass and buffelgrass on recordings

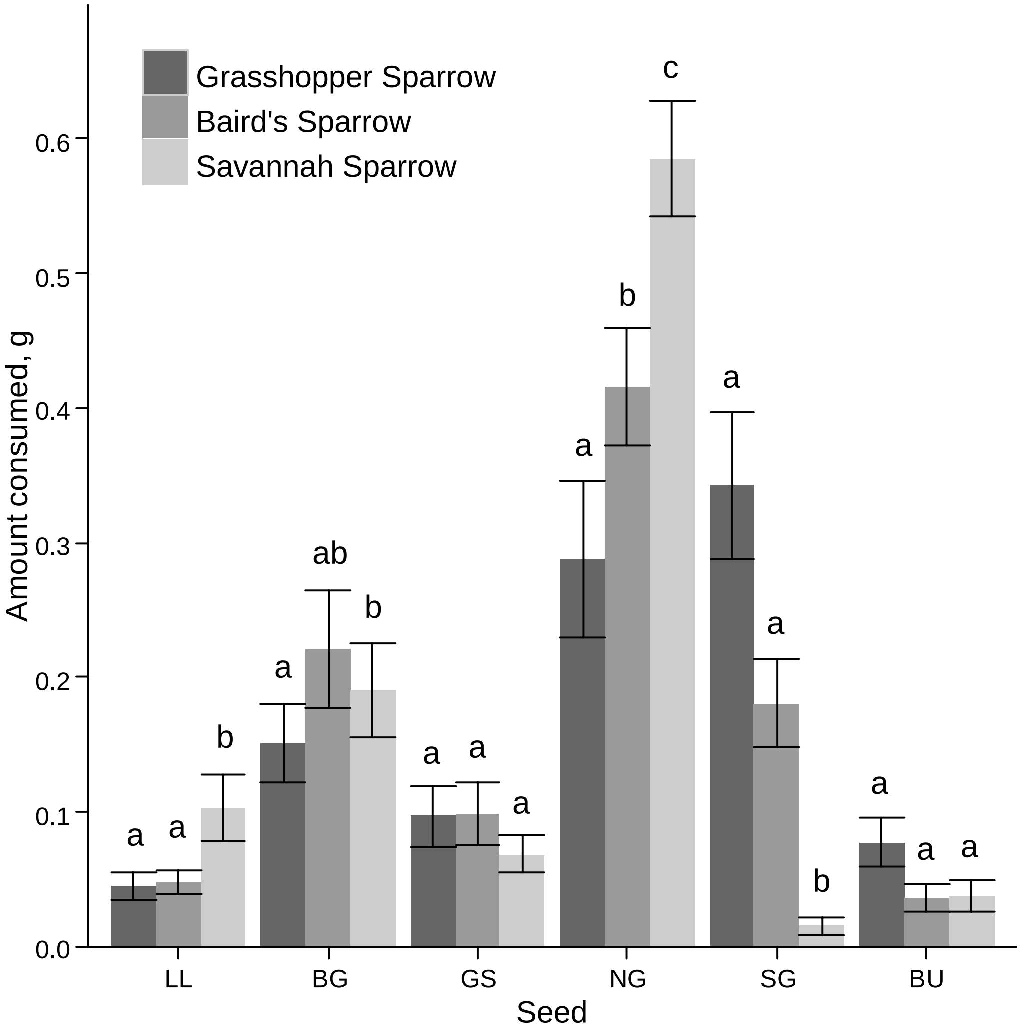

Figure 4. Amount consumed (± S.E.) per seed and bird species in the choice trials. Letters indicate significant differences ($P < 0.05$) between bird species based on a comparison of the regression coefficients in the Dirichlet regression. Seed species are order of size from small to large. LL = Lehmann lovegrass, BG = blue grama, GS = green sprangletop, NG = natal grass, SG = sideoats grama, BU = buffelgrass. Grasshopper sparrow = *Ammodramus savannarum*, Baird's Sparrow = *A. bairdii*, and Savannah Sparrow = *Passerculus sandwichensis*.

6, $P < 0.001$). This model was taken for interpretation of the regression coefficients to explore differences in consumption rates per seed species between bird species (Table 6). It shows that the largest differences in seed consumption patterns are found between Grasshopper and Savannah Sparrows (Figure 4), the two species that differ most in bill size (Table 4). Baird's Sparrow only differs significantly from Grasshopper Sparrow in the consumption of natal grass, and from Savannah Sparrow in the consumption of natal grass, sideoats grama and Lehmann love grass (Figure 4). All these differences are in the expected direction with respect to bill size of the species. For natal grass, the consumption followed the pattern of bill size. Savannah Sparrows consumed significantly more natal grass seeds than the other two sparrows, while Baird's Sparrows, with intermediate bill sizes, consumed significantly more than Grasshopper Sparrows. The pattern was reverse for sideoats grama, where Grasshopper Sparrows, with the largest bills, consumed more than Savannah and Baird's Sparrows, and Baird's Sparrows consumed more than Savannah Sparrows. Furthermore, Savannah Sparrows consumed significantly more Lehmann lovegrass, which were the smallest seeds, than Baird's and Grasshopper Sparrows but significantly less blue grama than the other two birds. There was no significant difference between the birds for the consumption of green sprangletop or buffelgrass seeds.

Consumption of seeds by bird species seems more uniform across seed species in the nonchoice trials (Figure 5) than in the choice trials (Figure 4). Nevertheless, the model including the interaction between seed and bird species (Table 7) was significantly better than a model without the interaction (likelihood-ratio test, $\chi^2 = 32.27$, df 20, $P < 0.001$), indicating that bird species differed in the

Table 6. Statistical results of the Dirichlet regression model with bird species as a factor and day as a covariate comparing consumption of the six seeds between Baird's (BAIS), Grasshopper (GRSP) and Savannah Sparrow (SAVS)[1] in the choice trials

Blue grama (*Bouteloua gracilis*)				
	Estimate	SE	Z[2]	P-value
BAIS vs. GRSP	0.3994	0.2806	1.423	0.155
SAVS vs. GRSP	0.7508	0.2837	2.647	0.008*
SAVS vs. BAIS	0.3514	0.2698	1.303	0.193
Green sprangletop (*Leptochloa dubia*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	0.0967	0.2875	0.336	0.737
SAVS vs. GRSP	0.1901	0.2941	0.646	0.518
SAVS vs. BAIS	0.0934	0.2857	0.327	0.744
Sideoats grama (*Bouteloua curtipendula*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	-0.3631	0.2816	-1.289	0.197
SAVS vs. GRSP	-1.4475	0.2943	-4.918	<0.001*
SAVS vs. BAIS	-1.0845	0.2919	-3.716	0.000*
Buffelgrass (*Pennisetum ciliare*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	-0.3312	0.2921	-1.134	0.257
SAVS vs. GRSP	-0.1364	0.2983	-0.457	0.648
SAVS vs. BAIS	0.1948	0.2918	0.668	0.504
Lehmann lovegrass (*Eragrostis lehmanniana*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	0.1917	0.2899	0.661	0.508
SAVS vs. GRSP	0.9905	0.2929	3.381	0.001*
SAVS vs. BAIS	0.7988	0.2799	2.854	0.004*
Natal grass (*Melinis repens*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	1.0307	0.2849	3.618	<0.001*
SAVS vs. GRSP	2.2112	0.2780	7.953	<0.001*
SAVS vs. BAIS	1.1805	0.2640	4.471	<0.001*

[1] *Ammodramus bairdii*, *A. savannarum* and *Passerculus sandwichensis*, respectively

[2] Significance of the estimates is tested with a z-test, Z = z-score (test statistic)

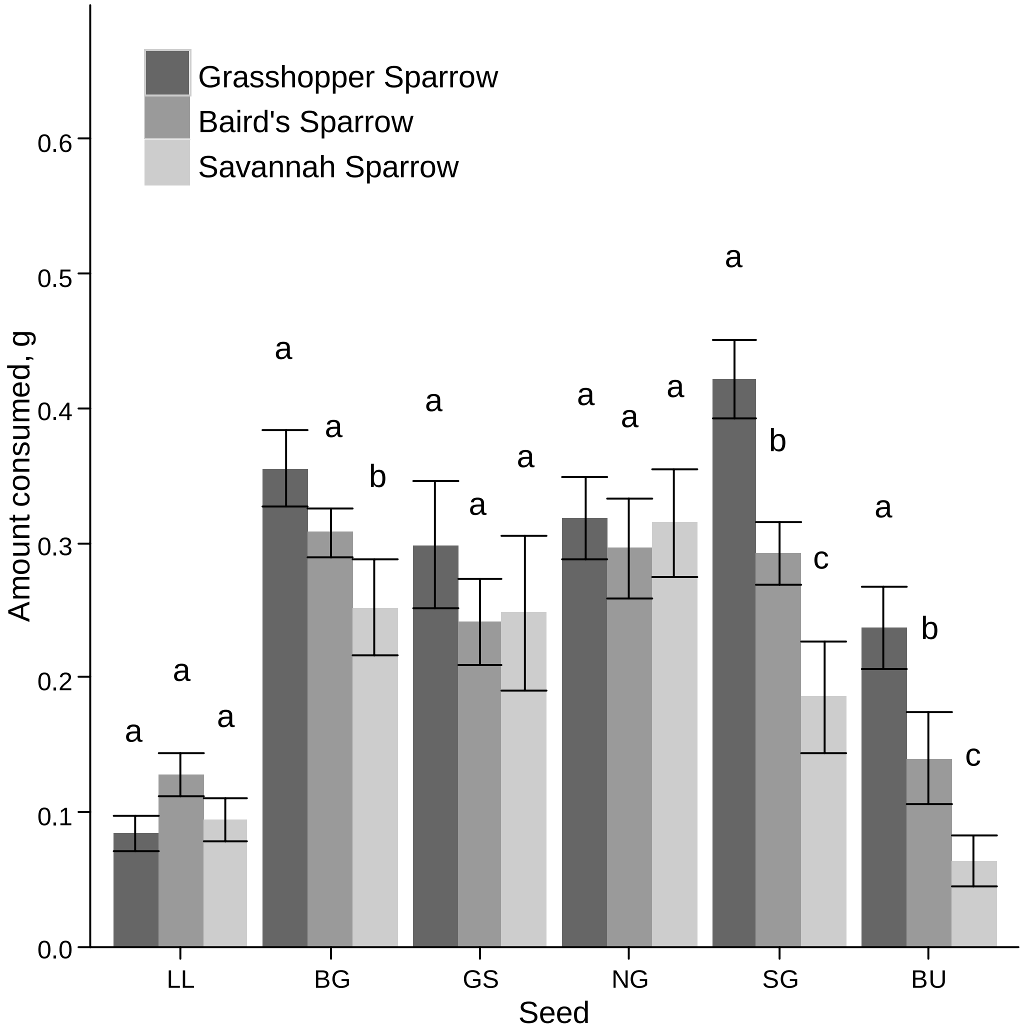

Figure 5. Amount consumed (± S.E.) per seed and bird species in the non-choice trials. Letters indicate significant differences ($P < 0.05$) between bird species based on pairwise comparisons for the linear mixed model. Seed species are order of size from small to large. LL = Lehmann lovegrass, BG = blue grama, GS = green sprangletop, NG = natal grass, SG = sideoats grama, BU = buffelgrass. Grasshopper Sparrow = *Ammodramus savannarum*, Baird's Sparrow = *A. bairdii*, and Savannah Sparrow = *Passerculus sandwichensis*.

Table 7. Results of the linear mixed model analysis for the nonchoice trials

Factor	df	SS	MS	F	P-value
Seed species	5	0.0890	0.1780	31.685	<0.001*
Bird species	2	0.1806	0.0903	5.092	0.017*
Seed species*Bird species	10	0.1819	0.0182	3.239	0.001*

consumption of at least one of the seed species. The results show that when they had no other choice, birds seemed to be able to profit from all seeds except for Lehmann lovegrass seeds and buffelgrass seeds (Figure 5). Specifically, Baird's and Savannah Sparrows consumed significantly less of these seeds than of all other seed species, while for Grasshopper Sparrows this was only true for Lehmann lovegrass seeds (all P < 0.05). Baird's Sparrows had an equal preference for all other seeds, while Savannah Sparrows also consumed fewer sideoats grama seeds compared to natal grass, blue grama and green sprangletop seeds (all P < 0.05). Grasshopper Sparrows consumed more sideoats grama and blue grama seeds compared to all other seeds, more natal grass compared to buffelgrass, and more buffelgrass and green sprangletop compared to Lehmann lovegrass (all P < 0.05). Pairwise comparisons for the differences between species (Table 8) indicate that birds differed significantly in the consumption of sideoats grama, following the same pattern as in the non-choice trials (Figure 5); Grasshopper Sparrows consumed more than Baird's and Savannah Sparrows and Baird's Sparrows consumed more than Savannah Sparrows. Furthermore, Grasshopper Sparrows consumed significantly more buffelgrass than Baird's and Savannah Sparrows, and significantly more blue grama than Savannah Sparrows (Figure 5)

We found that seeds of exotic grasses may be a source of food for passerine grassland birds wintering in the Chihuahuan Desert, provided that their size is adequate for birds' bill morphology. Natal grass seeds, with the most efficient handling time, were preferred. However, the two other exotic grass species, buffelgrass and Lehman lovegrass, were avoided. Preferences differed

Table 8. Pairwise comparisons of consumption of the six seeds between Baird's (BAIS), Grasshopper (GRSP) and Savannah Sparrow (SAVS)[1] in the nonchoice trials

Blue grama (*Bouteloua gracilis*)				
	Estimate	SE	Z[2]	P-value
BAIS vs. GRSP	-0.0476	0.0452	-1.051	0.293
SAVS vs. GRSP	-0.1031	0.0467	2.208	0.027*
SAVS vs. BAIS	-0.0556	0.0452	-1.229	0.219
Green sprangletop (Leptochloa dubia)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	-0.0567	0.0452	-1.253	0.210
SAVS vs. GRSP	-0.0500	0.0467	-1.070	0.285
SAVS vs. BAIS	0.0067	0.0452	0.1480	0.882
Sideoats grama (*Bouteloua curtipendula*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	-0.1288	0.0452	-2.848	0.004*
SAVS vs. GRSP	-0.2359	0.0467	-5.050	<0.001*
SAVS vs. BAIS	-0.1071	0.0452	-2.368	0.018*
Buffelgrass (*Pennisetum ciliare*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	-0.0968	0.0452	-2.141	0.032*
SAVS vs. GRSP	-0.1733	0.0467	-3.710	<0.001*
SAVS vs. BAIS	-0.0765	0.0452	-1.690	0.091
Lehmann lovegrass (*Eragrostis lehmanniana*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	0.0443	0.0452	0.980	0.327
SAVS vs. GRSP	0.0108	0.0467	0.232	0.817
SAVS vs. BAIS	-0.0335	0.0452	-0.741	0.459
Natal grass (*Melinis repens*)				
	Estimate	SE	Z	P-value
BAIS vs. GRSP	-0.0228	0.0452	-0.503	0.615
SAVS vs. GRSP	-0.0037	0.0467	-0.079	0.937
SAVS vs. BAIS	0.0197	0.0452	0.422	0.673

[1] *Ammodramus bairdii*, *A. savannarum* and *Passerculus sandwichensis*, respectively.

[2] Significance of the estimates is tested with a z-test, Z = z-score (test statistic)

between bird species. Since birds do not differ in body size, we can fairly assume that their energy requirements are comparable. Therefore, differences in seed preferences seem to be the result of variability in bill size, related to handling efficiency.

Handling times increased with seed size for all bird species, but more so for Savannah Sparrow, the species with the smallest bill. However, natal grass was an exception. Although this seed is bigger than three of the other seeds its handling time is shorter, thus it provides more energy over time compared to other seed species (i.e., it can be handled most efficiently). This may explain preferences for natal grass seeds observed in all birds. When birds had a free choice of seeds, they showed clear preferences for one or two seeds in particular. Grasshopper Sparrows, with the largest bill, preferred bigger seeds (sideoats grama) than Baird's and Savannah Sparrows that preferred natal grass seeds. However, Grasshopper Sparrows also consumed a considerable amount of natal grass seeds. When birds had no other choice they seemed to be able to consume most of the seeds except for Lehmann lovegrass and buffelgrass seeds. Therefore invasion of these grass species is probably an important threat to granivorous grassland birds. In this regard, breeding bird abundance has been found to be lower on grasslands invaded by buffelgrass and Lehmann lovegrass (Flanders *et al*., 2006). Our results suggest that a decrease in habitat carrying capacity due to the invasion of buffelgrass and Lehmann lovegrass could be even greater for wintering bird populations because, in contrast to breeding birds, they feed almost exclusively on seeds. However, seeds from invasive grasses are not necessarily unsuitable for the diets of granivorous birds (Larson *et al*., 2012), which is supported by the preference for natal grass seeds in this study.

Only Grasshopper Sparrows seemed able to exploit buffelgrass seeds. Buffelgrass seeds were the biggest seeds in this study and they have a husk with large awns that is difficult to manipulate (Figure 3). Few grassland passerine species may be able to meet their energy requirements by eating these seeds due to their large size. In line with this, birds with larger bills are able to profit from a wider range of seed sizes (Díaz, 1994; Desmond *et al*., 2008). In addition, Lehmann lovegrass seeds are so small that birds consuming these seeds may not be able to meet their energy requirements, at least for the range of bill sizes tested in this study. Chipping Sparrows (*Spizella passerina*) have been found to consume Lehmann lovegrass seeds in the field (Pulliam, 1986; Méndez-González, 2010). Chipping Sparrows have smaller bills, for which it could be possible that they are able to manipulate small seeds more efficiently than the larger-billed birds in this study (Díaz, 1990; Soobramoney and Perrin, 2007).

Natal grass seeds are of intermediate size but had the shortest handling time. The observed preference for these seeds suggests that seed selection in grassland passerines is a tradeoff between handling time and energy intake (seed size) which is in line with predictions from optimal foraging theory (which states that an animal will select those food items that maximize energy intake over time; Charnov, 1976). Alternatively, birds could simply be choosing seeds with the shortest handling time rather than maximizing energy intake over time (Thompson *et al*., 1987; Keating *et al*., 1992). However, the preference of Grasshopper Sparrows for sideoats grama contradicts the latter explanation, since sideoats grama seed had a longer handling time than some other seeds tested. Therefore, birds in this study might be foraging optimally. Indeed, optimal foraging theory is generally a good predictor of diets for species that forage on immobile prey such as seeds (Sih and Christensen, 2001).

In the choice trials, Savannah Sparrows mainly consumed natal grass seeds whereas the other two birds had a more variable diet. This might suggest that Savannah Sparrows are better able to rely on a single food source, one that is easily exploitable, whereas the other species require a more diverse array of seeds. This difference in diet might be an adaptation for exploiting a wider range of habitat types whereas *Ammodramus* spp. are restricted to native, intact grasslands (Desmond *et al*., 2005; Macías-Duarte *et al*., 2009).

Preferences for natal grass seeds may make birds effective agents in controlling the spread of this invasive grass species. Abundance of seeds preferred by granivorous birds decreases in the soil seed bank during the winter (Pulliam, 1986; Cueto *et al*., 2006; Desmond *et al*., 2008). However, preferred grass seeds in the Monte desert in Argentina did not result in a lower recruitment of these grasses in the next growing season (Marone *et al*., 2008). Monitoring of overwintering grassland birds in Mexico has mainly focused in grassland priority conservation areas (GPCAs) (Macías-Duarte *et al*., 2011). Natal grass is widespread in some GPCAs with the highest bird densities (personal observation), suggesting that the presence of natal grass does not negatively affect grassland bird density in winter. However, the abundance of exotic grasses in GPCAs in relation to bird densities has not been analyzed. Although natal grass seeds are apparently no problem for winter bird survival, there may be other habitat characteristics of invaded grasslands that make them unsuitable, such as a lack of plant species diversity, suitable near-ground vegetation structure or shrub cover. We recommend that future grassland bird monitoring efforts make a more explicit attempt at determining the effect of exotic grasses on species-specific bird densities in winter.

CONCLUSIONS AND RECOMMENDATIONS

Invasions of natal grass and Lehmann lovegrass are expected to increase as a result of climate change, as both species are drought resistant and have a higher seed production capacity than native grasses under regular as well as extreme weather conditions (Anable *et al*., 1992; Stokes *et al*., 2011; Melgoza-Castillo *et al*., 2014). These species often form monocultures, replacing native grasses, which may be detrimental to grassland birds. Therefore, it is important to educate private landowners on how to improve management practices to avoid extensive invasions as well as how to implement practices to actively control the spread of exotics. Since invasions of exotic grasses reduce the forage quality of pastures compared to blue grama grasslands (Melgoza-Castillo and Morales-Nieto, 2013), this will be in the interest of landowners as well.

Buffelgrass is often seeded and not considered invasive, although it has spread from ranches along roadsides. Some agricultural extension agencies are aggressively promoting planting of buffelgrass on rangelands, and this could have serious long-term consequences for grassland birds and other granivorous species. For example, SAGARPA, Mexico´s federal agricultural agency, is currently promoting a program to seed buffelgrass in a potential area that extends to over 6 million hectares through Mexico (SAGARPA, 2010). Therefore, action should be taken to discourage planting of exotic grasses, especially buffelgrass.

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STUDY 3: WINTER DIET OF BAIRD'S SPARROW (*Ammodramus bairdii*) AND GRASSHOPPER SPARROW (*Ammodramus savannarum*) IN NORTHERN MEXICO

RESUMEN

DIETA INVERNAL DE *Ammodramus bairdii* Y *Ammodramus savannarum* EN EL NORTE DE MÉXICO

POR:

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En este estudio se analizó la dieta de *Ammodramus bairdii* y *A. savannarum* en tres sitios y periodos en el desierto Chihuahuense en el norte de México. Las semillas del contenido de estómago regurgitado fueron identificadas con DNA barcoding utilizando el bucle P6 del intrón *trnL* como marcador genético. En cada muestreo se tomaron muestras aleatorias de suelo para estimar la disponibilidad de semillas. La resolución del marcador genético no fue suficiente para la identificación de semillas a nivel de especies y en ciertos casos a nivel de género. En el último caso, los datos fueron analizados a nivel de familia y los demás a nivel de género. Las dietas contenían una alta diversidad de semillas pero fueron dominadas por un número limitado. Las semillas de Panicoideae fueron las más comunes en las dietas (53 ± 19 %), seguido por *Bouteloua* spp. (10 ± 12 %). Dependiendo del sitio y periodo de muestreo, otras semillas importantes fueron Eragrostideae, *Pleuraphis* spp., Asteraceae, *Verbena* spp. y *Amaranthus* spp. Las semillas más comunes no siempre fueron las preferidas.

Aristida spp. y *Chloris* spp. fueron comunes en el suelo pero fueron evitados por ambas aves. Tanto *A. bairdii* como *A. savannarum* no difirieron en su selectividad, posiblemente porque son de tamaño similar. La importancia de Panicoideae y *Bouteloua* spp. en las dietas en todos los sitios y periodos indica la importancia de estas semillas para la sobrevivencia de *A. bairdii* y *A. savannarum* y, por lo tanto, la necesidad de prácticas de manejo que favorecen la producción de semillas de estos zacates y conservar pastizales medianos abiertos.

ABSTRACT

WINTER DIET OF BAIRD'S SPARROW (A*mmodramus bairdii*) AND GRASSHOPPER SPARROW (A*mmodramus savannarum*) IN NORTHERN MEXICO

BY:

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This study analyzed the diet of Baird's Sparrow (*Ammodramus bairdii*) and Grasshopper Sparrow (*A. savannarum*) in three different sites and sampling periods across the Chihuahuan Desert in northern Mexico. Seeds in regurgitated stomach contents were identified with a DNA barcoding approach using the P6 loop of the *trnL* intron as genetic marker. During each sampling period random soil samples were collected to estimate seed availability in the soil seed bank. The resolution of the genetic marker was too low to identify seeds at the species level and in some cases discrimination at genus level was neither possible. In the latter case seeds were analyzed at family level, otherwise at genus level. Diets contained a high variety of seeds but were dominated by only a limited number. Seeds from Panicoideae contributed for the largest part to the diets (53 ± 19 %), followed by *Bouteloua* spp. (10 ± 12 %). Depending on the site and sampling period other important seeds in the diets were Eragrostideae, *Pleuraphis* spp., Asteraceae, *Verbena* spp., and *Amaranthus* spp. Common seeds were not always preferred. *Aristida* spp. and *Chloris* spp. were common in the soil seed bank but these seeds were avoided by both bird species. Baird's and Grasshopper Sparrows did not differ in selectivity, possibly because they are very similar in size. The importance of Panicoideae and *Bouteloua* spp. in the diets

across all sites and sampling periods indicates that these seeds are important for winter survival of Baird’s and Grasshopper Sparrows and therefore the need for management practices to favor seed production of these grasses and conserve semi-open grasslands.

INTRODUCTION

Many grassland birds are migratory and over-winter in desert grasslands in Mexico. These grassland birds are among the most threatened in the world (Brennan and Kuvlesky, 2012). Declining availability of winter habitat is a main factor threatening grassland birds (Pool *et al*., 2014). To protect birds and conserve their habitat it is necessary to know more about their ecology, such as how they select winter grounds and how they move within their winter grounds. Habitat suitability and movement patterns are likely related to resource abundance and distribution (Ginter and Desmond, 2005). Granivorous grassland birds feed almost exclusively on seeds during winter (Desmond *et al*., 2008). In this regard, grassland bird abundance is positively associated with seed abundance (Dunning and Brown, 1982; Grzybowski, 1983; Bechtholdt and Stouffer, 2005). Grassland birds have been associated with vegetation characteristics such as grass cover, shrub cover, presence of forbs or grass height (Macías-Duarte *et al*., 2009; Block and Morrison, 2010; Macías-Duarte and Panjabi, 2013; Martínez-Guerrero *et al*., 2014; Henderson and Davis, 2014). However, little information exists on the actual diet composition and diet flexibility. Information on diet provides further means to evaluate habitat quality of grasslands throughout the Chihuahuan Desert, and explain patterns in abundance and distribution of grassland birds.

The diet of Chipping sparrows (*Spizella passerina*) in the southeastern grasslands of Arizona consists mainly of Lehmann lovegrass (*Eragrostis lehmanniana*), amaranth (*Amaranthus retroflexus*) and purselane (*Portulaca* spp.), while they prefer unarmored forb seeds over armored grass seeds (Pulliam, 1980). A comparison of the winter diets of five sparrows in southwestern New Mexico showed that dropseed (*Sporobolus* spp.) is preferred by Chipping sparrow, Brewer's sparrow (*Spizella breweri*) and Vesper sparrow (*Pooecetes gramineus*), and is important in the diet of Savannah (*Passerculus sandwichensis*) and White-crowned sparrow (*Zonotrichia leucophrys*) as well (Desmond *et al*., 2008). Other frequently consumed seeds were feather fingergrass (*Chloris virgata*), stinkgrass (*Eragrostis cilianensis*), amaranth (*Amaranthus* spp.) and carpetweed (*Mollugo verticillata*), with differences between bird species depending on their body size and differences among sites depending on the seeds available (Desmond *et al*., 2008). In both studies, sparrows expanded their diet towards the end of the winter by including a larger variety or less preferred seeds in their diet, supposedly as a response to the decrease of preferred seeds in the soil seed bank (Pulliam, 1980; Desmond *et al*., 2008).

Sparrow species in the Monte Desert of Argentina prefer grass over forb seeds with differences in selectivity between bird species (Cueto *et al*., 2006; Marone *et al*., 2008). The most important seeds in the diet of these sparrows include the grass seeds of sand dropseed (*Sporobolus cryptandus*), pappusgrass (*Pappophorum* spp.), streamed bristlegrass (*Setaria leucopila*), false Rhodes grass (*Trichloris crinita*), Arizona cottontop (*Digitaria californica*) and green sprangletop (*Diplachne dubia*; Marone *et al*., 2008). The only forb that represented and important fraction of the diet of one bird species was *Chenopodium papulosum* (Marone *et al.,* 2008). Preferences for grass seeds over forb seeds in these birds are likely related to a high starch content of grass seeds and the presence of toxic components in forb seeds (Ríos *et al*., 2012). Also here, selectivity resulted in a decrease of preferred species in the soil seed bank over time (Marone *et al*., 2008).

These studies show that birds do not consume seeds in relation to availability but that they are selective. Optimal foraging theory suggests that animals select food items in such a way as to maximize energy intake over time (Charnov, 1976). In this regard, laboratory studies show that the main determinant of seed selection appears to be seed size in such a way that birds select seeds that they can handle most efficiently (Pulliam, 1985; Díaz, 1996). Larger-billed birds are able to handle a wider range of seed sizes (Benkman and Pulliam, 1988; Keating *et al*., 1992) and this is reflected in the breadth of the diet, which is wider in larger birds than smaller birds (Desmond *et al*., 2008). Other determinants of seed selection may be energy content (Valera *et al*., 2005) or content of fat (Thompson *et al*., 1987; Molukwu *et al*., 2011), protein (Larson *et al*., 2012, Johansen *et al*., 2014), carbohydrates (Ríos *et al*., 2012), water (Carillo *et al*., 2007), or toxicity (Ríos *et al*., 2012).

However, selectivity is also determined by seed abundance and the spatial distribution of seeds in nature (Pulliam, 1986). In a situation with high seed abundance, birds may be more selective, specializing on a limited number of preferred seeds, whereas in a situation of low seed abundance birds may expand their diet and include less preferred seeds (Pulliam, 1985). Rainfall is the most important determinant of variability in seed production between years (Pulliam and Brand, 1975). However, abundance and diversity of plant species are the most important determinants of potential seed production and therefore seed abundance and diversity comparing sites. In this regard, grassland bird abundance and diversity is higher in sites with more dense and diverse vegetation (Desmond *et al*., 2004; Macías-Duarte *et al*., 2009). Additionally, bird abundance and seed abundance are positively related (Pulliam and Parker, 1979; Grzybowski, 1983; Ginter and Desmond, 2005). Therefore, which seeds are consumed and how selective birds are may vary between years and sites.

The present work studied the diet of Baird's Sparrow (*Ammodramus bairdii*) and Grasshopper Sparrow (*Ammodramus savannarum*) under natural conditions in three different time periods and sites across the Chihuahuan desert. Baird's and Grasshopper Sparrows are two declining sparrow species that are frequently associated (Desmond *et al.*, 2005). Both require good condition grasslands with tall grasses and dense cover (Macías-Duarte *et al*., 2009). These birds have comparable body morphology but differ in bill size, with Grasshopper Sparrow having a larger bill than Baird's Sparrow, possibly influencing differences in seed selection between the two species.

In contrast to previous studies, in the present study a DNA barcoding approach was used to identify seed species in regurgitated stomach and crop content. The use of DNA barcoding to identify diet components is more objective than identification of seed parts under a microscope and allows for the identification of very small pieces that cannot be identified using a microscope (Valentini *et al*., 2009a; Pompanon *et al*., 2012). For plant species there does not exist one established barcode, but several regions have been proposed. These include a combination of *matK* and *rbcL*, both in a coding region of the chloroplast DNA (Cowan and Fay, 2012), the intergenic spacer *trnH-psbA* (Hollingsworth *et* *al*., 2011), a short chloroplast region called the P6 loop of the *trnL* intron (Taberlet *et al*., 2007), and finally the nuclear regions nrITS and its shorter variant nrITS2 (Hollingsworth *et al*., 2011). Of these available barcodes, the P6 loop of the *trnL* intron is a small fragment that has been successfully used in several herbivore diet studies and was found to have a good performance with highly degraded DNA (Pegard *et al*., 2009; Valentini *et al*., 2009b; Soininen *et al*., 2013). Therefore, the P6 loop of the *trnL* intron was chosen to be used as a barcode in the present study, in which the DNA extracted from the regurgitated stomach samples resulted of low quality. Additionally, the DNA from stomach samples is contaminated with bird DNA for which a chloroplast barcode was desirable.

The expectation was that birds are selective in their diet and do not consume seeds in relation to availability. If birds would consume seeds in relation to their availability that would imply that birds walk randomly through the grassland and consume seeds as they are encountered without making any distinctions. This is unlikely and indeed, previous studies show that birds are selective in their diet and do not consume seeds in similar proportions as available (Pulliam, 1980; Desmond *et al*., 2008; Marone *et al*., 2008). It was also expected that Baird’s and Grasshopper Sparrow are selective in a different way leading to differences in their diet within sites.

MATERIALS AND METHODS

Study Sites

The study took place at three different locations in the Chihuahuan Desert, Mexico: Ecological Reserve “El Uno” (30º51’34” N, 108º27’17” W), the experimental ranch of the Autonomous University of Chihuahua “Teseachi” (28º32’38” N, 107º26’45” W), both in the state of Chihuahua, and a private ranch “Santa Teresa” (26º17’55” N, 10º09’54” W) in the state of Durango. El Uno is an ecological reserve dominated by *Bouteloua* spp. and *Aristida* spp. (Appendix A) in which grasslands are grazed by bison (*Bison bison*). Teseachi is dominated by *Bouteloua gracilis* (Appendix A) and is grazed by cattle using a rotational grazing system. Santa Teresa is a private ranch that is dominated by *Bouteloua* spp. and *Pleuraphis mutica* (Appendix A) and grazed by cattle and horses. In each site, sampling took place in semi open grasslands. Sites were characterized using 50 m vegetation transects during the first sampling period, and for every site a reference collection was constructed by taking one individual of all plant species encountered. Summer precipitation has been correlated to seed production (Pulliam and Parker, 1979) and sparrow abundance (Dunning and Brown, 1982), and was determined by calculating total rainfall in mm from May to October. Rainfall data were obtained from nearby weather stations of INIFAP, and the mean of 2 to 4 surrounding weather calculated to obtain precipitation data for each site. In January 2014, an additional pasture with Grasshopper Sparrows was sampled within El Uno because there were no Baird’s Sparrows that year but more Grasshopper Sparrows than the previous winter.

Data Collection

Each of the sites was sampled three times: November 2012, January 2013 and January 2014. Birds were caught using mist-nets that were placed in (semi-) open grassland areas within the study sites. Baird's and Grasshopper Sparrows were banded using USGS bands and the following measurements were taken: weight (to the nearest 0.1 g), wing and tail length (mm) using a ruler, molt, age, tarsus (to the nearest 0.1 mm), bill length, width and depth (to the nearest 0.1 mm) using a calliper, and fat (on a scale from 0 to 5). Stomach samples were taken following the method of Desmond *et al.* (2008). Briefly, a catheter was inserted into the stomach of the bird after which warm water was injected using a syringe until the birds regurgitated their stomach content. Birds were released immediately after the sample was taken. The stomach content was collected on a coffee filter and dried immediately. Stomach samples were saved in manila envelopes until further analysis in the laboratory. In November 2012 and January 2013 several stomach samples were obtained from Savannah Sparrows (*Passerculus sandwichensis*) and Vesper Sparrows (*Pooecetes gramineus*) as well. These samples were used to standardize laboratory protocols (see below).

Soil Sampling

In each of the sites, random soil samples were taken to estimate the seed abundance and diversity in the soil. A minimum of 25 soil samples were taken per sampling location in every sampling period. Seeds were separated from soil using a maze and identified and counted under a microscope. Biomass availability was calculated for the most common seed species analyzed (see below). Seed mass data were provided by César Méndez (personal communication) or obtained from literature (Pulliam, 1980; 1985; 1986; Méndez-González, 2010).

Laboratory Analysis

Initially, the goal was to identify the seeds in the regurgitated stomach samples using a microscope, following Desmond *et al.* (2008) and Pulliam (1980). However, the seeds came in very small pieces that were mostly unidentifiable and therefore, it was decided to use a molecular approach. DNA Barcoding has successfully been used in several diet studies (*e.g*., Pegard *et al.,* 2009; Valentini *et al*., 2009; Soininen *et al.*, 2013) and has been recommended as a more objective way of diet analysis from stomach content or feces (Pompanon *et al.*, 2012).

DNA extraction. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturers protocol. Samples were prepared for extraction under liquid nitrogen using a mortar and pestle. Nitrogen was not poured directly onto the sample. Rather, the mortar was placed inside the nitrogen and the sample was allowed to freeze before further processing. Extraction followed immediately after this and samples were not allowed to thaw. In some cases, a micropestle was used to grind the sample further inside the tube after the buffer had been applied in the first step of the extraction protocol. This was only done in case large pieces of seed were still visible after vortexing. This sample preparation method resulted in the best DNA concentrations following several tests with samples from Savannah and Vesper Sparrows, using different methods, including a mortar and pestle, a micropestle to grind samples in the microcentrifuge tubes, and direct or indirect nitrogen application. Samples were combined to obtain a sufficient amount for extraction by grouping 3-5 samples of the same bird species in one site and sample period. Final elutions were performed in 50 µl to obtain a higher concentration. The second elution was performed in a separate microcentrifute tube. DNA concentration was measured using Nanodrop and DNA was stored at -20 °C.

DNA amplification and sequencing. The barcode used for seed identification was the P6 loop of the *trnL* (UAA) intron (Table 1) using primers *g* and *h* from Taberlet *et al.* (2007), which is a chloroplast region. This is important because samples are contaminated with bird DNA. PCR was performed in two steps during which identification tags and sequencer tags were attached to the sequences. Identification tags were designed for each bird species × study site × sampling period combination. The first amplification round involved 1 cycle of 10 min at 95 °C, 30 cycle

final cycle of 5 min at 72 °C, and wa

using 4 ng of DNA and 1 µL of each primer in addition to DMSO at a final concentration of 3

%. The results were verified with an agarose gel at 1 %. The product of the first PCR was purified using Agencourt AMPure XP Beads (Agencourt) and resuspended in a volume of 10 µL. After this the samples were pooled. To do this the concentration of each PCR product was quantified using a Qubit High Sensitivity Assay (Qubit) and pools were formed by combining an equal volume for every sample at a concentration of 1 nM. The second amplification round was performed in a volume of 25 µL using 5 µL of every pool, 2.5 µL Nextera XT Index Primer 1 and 2.5 µL Nextera XT Index Primer 2 (N7XX and S5XX, respectively; Illumina), 2.5 µL of water and 12.5 µL of 2X Phusion PCR Master Mix (Phusion).

The mixture was denatured at 98 °C for 30 s followed by 8 cycles of 10 s at 98 °C, 15 s at 55 °C and 15 s products were then purified using Agencourt AMPure XP Beads (Agencourt) and resuspended in a volume of 20 µL of eluation buffer. The DNA concentration in every pool was determined using a Qubit High Sensitivity Assay (Qubit) to prepare 4 nM of every pool. Pools were then mixed. The final concentration was determined using RT-PCR with the Universal KAPA Library Quantification Kit (KAPA Biosystems) for Illumina platforms. Samples were then sequenced with Illumina MiSeq (Illumina) using a kit for 300 cycles.

Twenty reference plant species were amplified and sequenced using the same barcode region. The PCR program involved 1 cylce of 10 min at 95 °C, 45 cycles of 30 s at 95 °C, 30 s at 5

5 min at 72 °C. Reference plants were sequenced by the Sanger method because only one sequence per sample needed to be obtained. The reference plants were selected based on their abundance in one or more study sites as detected in vegetation transects (Appendix A) or because they have been found to be common in the diet of related sparrow species (Desmond *et al*., 2008). These plants were: *Bouteloua gracilis*, *B. curtipendula*, *Botriochloa barbinoidis*, *Setaria macrostachya*, *Muhlenbergia rigida*, *Schkuria pinnata*, *Haplopappus gracilis*, *Panicum obtusum*, *Amaranthus palmeri*, *Eragrostis cilianensis*, *Aristida adscencionis*, *Chenopodium alba*, *Digitaria californica*, *Pleuraphis mutica*, *Chloris virgata*, *Enneapogon desvauxii*, *Mollugo verticillata*, *Sporobolus airoides*, *Portulaca pilosa*, and *Lycurus phleoides*.

Sequence analysis. Sequences were identified by comparison to the sequenced reference collection as well as a customized database constructed by taking the target sequences from all plants encountered in either the reference collection of plant species collected in the field, vegetation transects or soil samples from GenBank (NCBI). Including the reference collection, the customized database included 166,834 sequences. The alignment was performed using the program SMALT 0.7.6. Because the genetic barcode did not always discriminate well between species, data were analyzed per genus. The number of reads per genus was calculated for each group (bird species × study site × sampling period) as well as the proportion of the total number of reads per genus.

The resolution of the genetic barcode proved insufficient to discriminate reliably between seed species and in some cases between genera. The latter was the case for the families of Panicoideae, Eragrostideae and Asteraceae. Sometimes birds would appear to be consuming seeds that were not present at a site based on the three different characterization methods (soil samples, vegetation transects and reference collection). However, a related seed was usually present at those sites. To overcome problems with the resolution of the barcode, seeds of Panicoideae (*Botriochloa* spp., *Eriochloa* spp., *Hackelochloa* spp., *Panicum* spp., and *Setaria* spp.), Eragrostideae (*Eragrostis* spp., *Lycurus* spp., and *Muhlenbergia* spp.) and Asteraceae (*Hypochaeris* spp., and *Machaeranthera* spp.) were analyzed by families and other seeds by genus.

Statistical Analysis

To investigate whether birds are selective in their diet, a Dirichlet regression was used with diet composition as the dependent variable and sample (diet or soil) as independent variable. A Dirichlet regression is a type of compositional analysis based on the beta distribution and does not assume a multivariate normal distribution or homoscedasticity of the data (Maier, 2014). As all compositional analyses, it works on a log-transformation of the compositional variable. This transformation overcomes potential problems with non-independence of proportional data (Aebischer *et al*., 1993). Because of this transformation, it is not possible to have zeros in the data, therefore they were replaced by a small value (Aebischer *et al.*, 1993). Because in some cases the proportion of reads was smaller than 0.001, zero values were replaced by 0.0001. The compositional response was based on the most common seeds in either diet or soil samples. The criteria used to select these seeds was an abundance of 10 % or more in at least one group (bird species × study site × sampling period) or soil in one sampling period.

To test the hypothesis that selectivity differs between bird species, a Multivariate Analysis of Variance (MANOVA) was performed on the log-ratios (difference between the log-transformed proportion in the diet and the log-transformed proportion in the soil) for the selected seeds, with bird species as factor, and precipitation as a covariate. Samples were taken in different study sites and sampling periods. The main interest here was to sample the diets in their widest extent possible, not in differences between sites and sampling periods per se. However, study site may encompass several ecological or environmental variables that could influence the diets. Precipitation, which was measured, is only one of these variables. Other important variables could, for example, be related to differences in vegetation characteristics between sites. To control for the effects of these unmeasured variables, study site and sampling period were added as factors to control for variability between sites and sampling periods. Thus, the full model was:

Log-ratio diet/soil = BIRD SPECIES + STUDY SITE + SAMPLING PERIOD + precipitation

Wilk's ʎ was used as test statistic. The final model was obtained through backward deletion of non-significant terms. The assumption of multivariate normality of the residuals was checked graphically.

One seed dominated the diets in all sites. To test whether the proportion of the most common seed in the diet is influenced by biomass availability of the most common seeds in the soil and differs between bird species, an Analysis of Variance (ANOVA) was performed with proportion of total reads of the most common seed as dependent variable, bird species and study site as well as their interaction as factors, and biomass availability of the most common seeds in the soil as covariates:

Proportion of total reads = BIRD SPECIES + STUDY SITE + BIRD SPECIES × STUDY SITE + biomass availability

The final model was obtained through backward deletion of non-significant terms. The assumption of normal distributed residuals was checked graphically and using a Shapiro-Wilk normality test.

Previous studies have found a decrease in seed abundance of preferred seeds from early to late winter (Pulliam *et al*., 1986; Desmond *et al*., 2008; Marone *et al*., 2008). To test whether this was true, the data of the first season (sampling period 1 and 2) were analyzed using three different ANOVAs with biomass of the preferred seeds, biomass of the most common seeds or biomass of the single most common seed as response variables and sampling period (November 2012: early winter, January 2013: mid winter) as factor as well as study site to control for its effect:

(1) Biomass preferred seed = SAMPLING PERIOD + STUDY SITE

(2) Biomass most common seeds = SAMPLING PERIOD + STUDY SITE

(3) Biomass single most common seed = SAMPLING PERIOD + STUDY SITE

RESULTS AND DISCUSSION

In total, 146 Baird's Sparrow samples and 182 Grasshopper Sparrow samples were collected. Mist-netting efforts indicated that bird abundance differed between the years. Generally, the winter of 2013-2014 was milder with more previous summer rainfall (Table 9) which was reflected in bird abundance. However, Santa Teresa received less summer rainfall preceding the second sampling season and that year the ranch was heavily grazed for which we encountered fewer birds and no Grasshopper Sparrows. In El Uno, bird abundance was much higher in January 2014 as compared to the other sampling periods; but strangely, there were no Baird's Sparrows whereas the previous year there were individuals of Baird's Sparrow. In Teseachi, grassland condition was good both winters and both species were found in all three sampling periods. However, Baird's Sparrows were less abundant during the first winter. Table 10 shows how many samples were collected per site and sampling period combination.

Diet Samples

Sequencing results show that the main seeds consumed by Baird's and Grasshopper Sparrows in all sites belonged to the family of Panicoideae, as these seeds represented from 10 to 84 % of total reads in the diet samples, depending on site, sampling period and bird species (Table 11; Appendix B). Other commonly consumed seeds in all sites were *Bouteloua* spp., ranging from 0 to 35 % of total reads (Table 11; Appendix B). For other seeds, consumption was more variable between study sites, sampling periods and bird species. Next to Panicoideae and *Bouteloua* spp., *Pleuraphis* spp. and Eragrostideae were

Table 9. Precipitation (mm) from May to October preceding the two sampling seasons (winter of 2012-2013 and 2013-2014)

	Santa Teresa[1]	Teseachi[2]	El Uno[2]
Season 1	361.75	317.15	194.81
Season 2	301.25	472.43	260.33

[1] Durango, Mexico.
[2] Chihuahua, Mexico.

Table 10. Collected stomach samples in Chihuahua and Durango, Mexico, per bird species, study site and sampling period

	Baird's Sparrow[1]			Grasshopper Sparrow[2]		
	Teseachi	El Uno	Santa Teresa	Teseachi	El Uno	Santa Teresa
Nov 2012	12	13	44	36	18	11
Jan 2013	7	15	21	27	22	19
Jan 2014	21	0	13	16	33	0

[1] *Ammodramus bairdii*
[2] *A. savannarum*

Table 11. Most common seeds in diet samples (mean percentage of total sequence reads ± S.D.) from Baird's (BAIS) and Grasshopper Sparrow (GRSP)[1] per site, averaged over the sampling periods (n[2])

	Santa Teresa[3]		Teseachi[3]		El Uno – Centro[3]		El Uno – Lora[3]
	BAIS	GRSP	BAIS	GRSP	BAIS	GRSP	GRSP
	n = 3	n = 2	n = 3	n = 3	n = 2	n = 3	n = 1
Panicoideae	44.03	22.80	45.00	52.36	78.98	70.49	63.99
	(8.03)	(17.41)	(3.58)	(12.95)	(6.49)	(13.18)	(--)
Bouteloua spp.	4.07	33.31	15.21	10.76	3.29	0.28	5.90
	(2.60)	(1.95)	(13.00)	(7.94)	(4.50)	(0.36)	(--)
Pleuraphis spp.	16.67	8.15	5.17	3.95	2.65	6.33	3.48
	(12.30)	(1.10)	(1.16)	(1.24)	(3.54)	(3.52)	(--)
Eragrostideae	14.10	5.04	10.93	7.67	5.25	11.77	4.81
	(9.88)	(3.36)	(3.85)	(1.16)	(6.99)	(8.10)	(--)
Asteraceae	8.48	8.78	2.44	4.62	1.21	0.71	0.19
	(8.93)	(11.75)	(4.07)	(6.66)	(0.78)	(0.45)	(--)
Verbena spp.	0.66	0.05	8.38	4.38	0.02	0.03	0.00
	(1.02)	(0.06)	(12.98)	(6.94)	(0.02)	(0.04)	(--)
Amaranthus spp.	0.51	0.00	0.00	0.00	0.01	0.34	14.11
	(0.88)	(0.00)	(0.00)	(0.00)	(0.01)	(0.58)	(--)

[1] *Ammodramus bairdii* and *A. savannarum*, respectively.

[2] Diet samples were combined in pools for molecular analysis (see material and methods). All samples within one site and sampling period were combined for each species. Therefore it was not possible to determine between individual variability and n refers to the number of pools, here one for each sampling period.

[3] Durango and Chihuahua, Mexico, respectively.

common in Santa Teresa and El Uno. Eragrostideae were also frequently consumed in Teseachi, together with *Verbena* spp. (Table 11; Appendix B).

It should be noted that is an assumption to take the proportion of sequence reads to be equivalent to the actual proportion of a seed biomass in the diet. Bias may occur during the different processing steps of the samples, especially PCR amplifications, because a small difference in amplification efficiency between two different seed species may result in a large difference in the amount of sequence copies after several PCR cycles (Pompanon *et al*., 2012). Another source of error is variability between the seeds themselves in characteristics such as gene copy number or differences in the state of digestion (Pompanon *et al*., 2012), although the latter will also affect seed identification using a microscope. However, comparisons of sequence-based data with traditional identification methods suggest that the proportion of sequence copies is a reasonable reflection of the actual proportion of a food item in the diet (Soininen *et al*., 2009; Deagle *et al.*, 2010).

Soil Seed Bank

The soil seed bank contained up to 108 different seed species. On average, seed production was 1.5×10^9 seeds ha^{-1} for the first season and 6.4×10^9 seeds ha^{-1} for the second season. Seed production differed between sampling periods (F = 11.26, P = 0.006) but not between sites (F = 1.45, P = 0.355). *Post-hoc* comparisons show that seed production did not differ from early to mid-winter during the first season (Tukey HSD, November 2012 *vs*. January 2013: P = 0.959) but was higher for the second season (Tukey HSD, November 2012 *vs*. January 2014: P = 0.015; January 2013 *vs*. January 2014: P = 0.010). Although this was likely the result of the higher precipitation, precipitation did not correlate with seed production for total seeds per ha (R = -0.121, P =0.739) or for biomass availability of the most common seeds (R = 0.219, P = 0.543). Seed production was higher in 2014 for all sites, whereas precipitation was always higher in some sites compared to others (Table 9), irrespective of sampling period. This may explain the lack of a correlation between precipitation and seed production, because both sites with higher and lower precipitation had more seeds in the last sampling period. Previous studies found a reduction of seeds in the soil seed bank from mid-winter to late winter (Pulliam, 1986; Desmond *et al*., 2008; Marone *et al*., 2008). Here seed abundance did not differ within one season. However, in the present study seed abundance was measured in early (November) and mid-winter (January), in contrast to mid- (January) and late winter (March). This may explain why here seed abundance did not differ between sampling periods within one season whereas in other studies it did.

Common seeds in the soil seed bank of all sites were Panicoideae, *Aristida* spp., and *Bouteloua* spp. (Table 12, Appendix A). Interestingly, *Aristida* spp. were rarely found in the diet samples (Table 13; Appendix B). Other seeds differed between sites. In the last sampling period, an additional site was sampled in El Uno with a very high production of *Amaranthus* spp. Asteraceae were common in Santa Teresa but absent in Teseachi. Teseachi was the only site containing *Verbena* spp., and *Pleuraphis* spp. were only present in Santa Teresa and El Uno-Centro (Table 12, Appendix A).

Comparison of Diets with the Soil Seed Bank

A comparison of the proportion of seeds in the soil samples with the

Table 12. Mean (± S.D.) biomass (kg/ha) of the most common seeds in the soil seed bank that were used for analysis per site averaged over the three sampling periods

	Santa Teresa[1]	Teseachi[2]	El Uno – Centro[2]	El Uno – Ratones[2]
	n = 3	n = 3	n = 3	n = 1
Panicoideae	80.85	353.82	221.59	737.50
	(83.03)	(205.88)	(297.23)	(--)
Bouteloua spp.	105.60	69.33	127.05	499.97
	(22.54)	(20.24)	(132.61)	(--)
Pleuraphis spp.	29.85	0.00	15.11	0.00
	(13.58)	(0.00)	(21.74)	(--)
Eragrostideae	11.27	33.86	19.23	137.68
	(18.26)	(22.46)	(33.30)	(--)
Asteraceae	300.34	0.27	50.35	62.25
	(336.78)	(0.23)	(7.73)	(--)
Verbena spp.	0.00	16.42	0.00	0.00
	(0.00)	(13.43)	(0.00)	(--)
Amaranthus spp.	0.09	0.00	2.10	1111.68
	(0.15)	(0.00)	(1.05)	(--)
Chloris spp.	82.69	49.11	1.76	14.56
	(51.16)	(45.10)	(1.64)	(--)

[1] Durango, Mexico.
[2] Chihuahua, Mexico.

Table 13. Comparison of the average proportion (± S.D.) of the nine most common seeds in diets and soil seed bank based on their total

	Baird's Sparrow[1] n = 8		Grasshopper Sparrow[1] n = 9	
	Diet	Soil	Diet	Soil
Panicoideae	0.60 (0.18)	0.27 (0.21)	0.61 (0.23)	0.31 (0.19)
Bouteloua spp.	0.09 (0.10)	0.18 (0.10)	0.15 (0.18)	0.20 (0.08)
Pleuraphis spp.	0.10 (0.11)	0.03 (0.04)	0.07 (0.03)	0.03 (0.04)
Eragrostideae	0.12 (0.08)	0.02 (0.03)	0.09 (0.06)	0.03 (0.03)
Asteraceae	0.05 (0.07)	0.18 (0.16)	0.05 (0.09)	0.12 (0.12)
Verbena spp.	0.04 (0.09)	0.01 (0.02)	0.02 (0.05)	0.01 (0.02)
Amaranthus spp.	0.00 (0.00)	0.00 (0.00)	0.02 (0.05)	0.05 (0.13)
Chloris spp.	0.00 (0.00)	0.07 (0.05)	0.00 (0.00)	0.05 (0.05)
Aristida spp.	0.00 (0.00)	0.24 (0.16)	0.00 (0.00)	0.20 (0.16)

[1] *Ammodramus bairdii* and *A. savannarum*, respectively.

proportion of seeds in diet showed that birds did not consume seeds according to their abundance, with the exception of *Amaranthus* spp. (B = -0.25, P = 0.468) and Asteraceae (B = -0.31, P = 0.347; Figure 6). Panicoideae, *Pleuraphis* spp., Eragrostideae and *Verbena* spp. were consumed proportionally more than would be expected based on their availability in the soil seed bank (B = -1.18, P < 0.001; B = -1.85, P < 0.001; B = -1.73, P < 0.001; B = -0.70, P = 0.038, respectively; Figure 6). *Bouteloua* spp., *Chloris* spp., and *Aristida* spp., were proportionally consumed less than expected based on their availability (B = 0.60, P = 0.050; B = 0.99, P = 0.003; B = 2.53, P < 0.001, respectively; Figure 6). Diet composition did not differ significantly between bird species (F = 6.04, P = 0.306; Figure 6). In other words, Baird's and Grasshopper Sparrows were selective in their diet showing preferences for some seeds and avoidance of others, but they did not differ in their preferences. Previously it was found that Grasshopper Sparrows are able to exploit larger seeds than Baird's Sparrows, although there was a considerable overlap in preferences for other seed species (Study 2). Grasshopper Sparrows have slightly larger bills than Baird's Sparrows (Appendix C) for which it may be expected that they can profitably consume larger or harder seeds (Díaz, 1996; Van der Meij *et al*., 2004). However, seed size variability of seeds consumed in the field may be small overall, limiting the potential for seed size partitioning between Baird's and Grasshopper Sparrows (Benkman and Pulliam, 1988). Additionally, Baird's and Grasshopper Sparrow are in fact quite similar with respect to body and bill size compared to other sparrows (Méndez-González, 2010).

Although *Bouteloua* spp. appear to be avoided, they were actually the

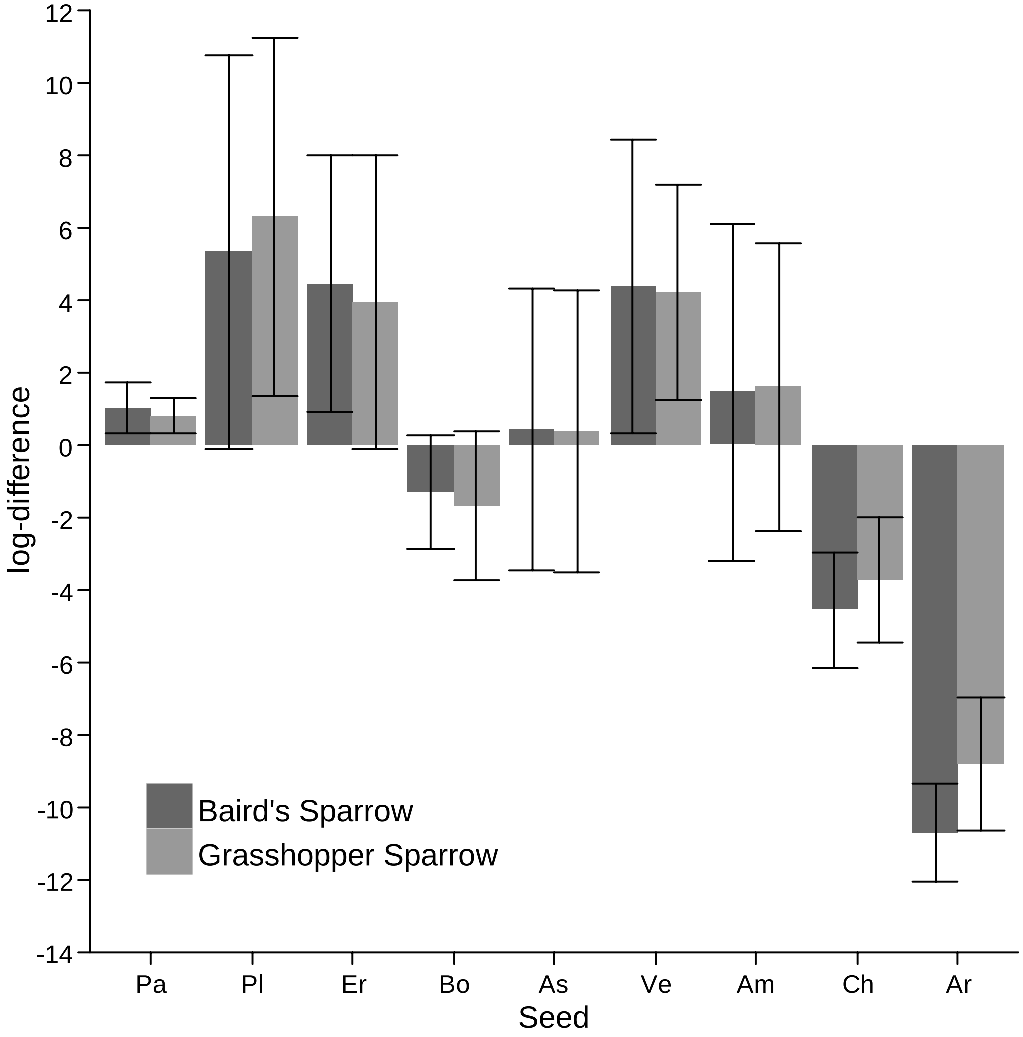

Figure 6. Log-ratios between seeds in diet and soil samples. A positive value means that the proportion in the diet is higher than in the soil, a negative value means that the proportion in the soil is higher than in the diet. Bars represent 95 % confidence intervals, indicating significant differences between diet and soil (i.e., 95 % C.I. does not include zero). (Pa = Panicoideae, Pl = *Pleuraphis* spp., Er = Eragrostideae, Bo = *Bouteloua* spp., As = Asteraceae, Ve = *Verbena* spp., Am = *Amaranthus* spp., Ch = *Chloris* spp., Ar = *Aristida* spp.). Baird’s Sparrow = *Ammodramus bairdii*, Grasshopper Sparrow = *A. savannarum*.

second most common seed in the diet on average, ranging from 0 to 35 % (Table 11), and in some cases even the most consumed seeds (Appendix B). *Bouteloua* spp. were also one of the most abundant seeds in the soil seed bank (Table 12). Thus, Baird’s and Grasshopper Sparrows are able to exploit *Bouteloua* spp. seeds when they are present, although they might prefer other seeds (in this case Panicoideae) if available. *Bouteloua* spp. were dominant in all study sites (Appendix A) and therefore *Bouteloua* spp. seeds may be too abundant for birds to consume them more than in proportion to their availability. This explanation is unlikely however, considering that biomass availability of other seeds in the soil seed bank was higher in some sites (Table 12). On the other hand, *Bouteloua* spp. seeds are smaller on average than for example the preferred Panicoideae seeds, therefore birds would need to consume more *Bouteloua* spp. seeds to reach the same amount in biomass. Possibly handling efficiency is lower for *Bouteloua* spp. seeds because they provide less energy over time than Panicoideae seeds. Considering that the unhusked seed masses of *Bouteloua* spp. and Panicoideae seeds are 0.48 and 0.86 g, on average, a bird would have to consume 1.8 *Bouteloua* spp. seeds for every Panicoideae seed. Whether it is more profitable to consume Panicoideae or *Bouteloua* spp. depends, however, on the handling time of the two seeds. Previously, it was found that Baird’s and Grasshopper Sparrows on average need 0.64 and 0.47 s to handle a *B. gracilis* seed and 0.89 and 0.66 s to handle a *B. curtipendula* seed, respectively (Study 2). Handling times for Panicoideae seeds are unknown for Baird’s and Grasshopper Sparrows. Chipping Sparrows need 1.3 s for *B. gracilis*, 1.4 s for *B. curtipendula vs*. 4.5 s for a *Panicum obtusum* seed (Pulliam, 1986). However, Chipping Sparrows are much smaller than Baird's and Grasshopper Sparrows and have smaller bills, and handling time is strongly related to bill size (Keating *et al*., 1992; Díaz, 1994; Soobramoney and Perrin, 2007; Study 2). Seed-eating birds have been found to prefer seeds that they can handle most efficiently as to maximize energy intake over time (Pulliam, 1985; Díaz, 1996; Hrabar and Perrin, 2002). It is possible that Baird's and Grasshopper Sparrows are able to increase energy intake over time by consuming more of the larger Panicoideae seeds compared to the smaller *Bouteloua* spp. seeds if these seeds can be handled relatively fast. The large amount of *Bouteloua* spp. seeds in Baird's and Grasshopper Sparrow diets suggests, however, that these seeds are important for their survival during the winter. In line with this, Baird's and Grasshopper Sparrows have previously been associated with *Bouteloua* spp. (Desmond *et al*., 2005). *Bouteloua* spp. are native to the Chihuahuan Desert grasslands and generally indicate a good quality grassland. Baird's and Grasshopper Sparrows require dense vegetation with tall grasses (Macías-Duarte *et al*., 2009). Thus, the vegetation characteristics associated with *Bouteloua* spp. might be more important for Baird's and Grasshopper Sparrow survival than their seeds, although the dominance of *Bouteloua* spp. in their diets shows that both bird species are able to exploit *Bouteloua* spp. seeds.

The results for Eragrostideae need to be interpreted with caution. Although a considerable proportion of the diet consisted of these seeds (0 - 25 %), these seeds were not detected in the soil in El Uno during the first winter. This could partly be related to sampling error, since Eragrostideae seeds are small and could have passed detection. Plants of this family were not found in the vegetation transects; although *Eragrostis cilianensis*, *E. lehmanniana* and *Muhlenbergia minutissima* were observed. Because the soil data for el Uno have zero abundance for Eragrostideae in the first two sampling periods, comparison of consumption with availability might show a larger preference than is real. The presence of these seeds in the diet shows that Baird's and Grasshopper Sparrow do consume the smaller Eragrostideae seeds as well. In a previous study, it was found that Baird's and Grasshopper Sparrows avoid seeds from the introduced *Eragrostis lehmanniana* (Study 2). The main Eragrostideae in the soil samples of the study sites were *E. cilianensis* and *Lycurus phleoides*. Husked seed masses of these three species are 0.1, 0.1, and 0.14 g, respectively, showing that they do not differ much in size. The reason that Baird's and Grasshopper Sparrows would avoid small Eragrostideae seeds is that they are likely not able to meet their energy requirements by consuming only these small seeds (Méndez-González, 2010). However, *E. cilianensis* was also common in the diet of other smaller- as well as larger-bodied sparrows (Desmond *et al*., 2008). Here, Eragrostideae seeds co-occured in the soil with the larger Panicoideae seeds. Possibly birds may be able to consume the smaller Eragrostideae seeds in this case since the larger Panicoideae seeds make it easier to satisfy energy requirements. If Eragrostideae seeds fall clumped together, this would also limit searching time, for which profitability increases.

Site and precipitation significantly affected the log-ratios between diet and soil composition (F = 14.40, P < 0.001; F = 6.73, P =0.041) and there was a tendency for sampling period to influence the log-ratios (F = 4.51, P = 0.077). However, seed availability differed greatly between sites (Table 12), which would result in differences in diet composition and selectivity (i.e., birds cannot consume seeds that are not present). Moreover, the log-ratios are zero in case of proportional consumption but also when seeds are not present (because they cannot be consumed). Indeed, univariate tests confirm that site is only significant for seeds that vary greatly in their availability between sites; Panicoideae (F = 4.87, P = 0.009), *Pleuraphis* spp. (F = 485.34, P < 0.001), Asteraceae (F = 118.49, P = 0.002), and *Verbena* spp. (F = 216.81, P < 0.001). In a similar way, the effect of precipitation can be explained by the strong relationship that precipitation has with site (Table 9). Looking at the univariate tests for precipitation, it can be seen that it has a significant effect for Asteraceae (F = 59.62, P = 0.003) only and a trend for *Verbena* spp. (F = 14.55, P = 0.099). Teseachi has the highest precipitation in sampling period 3 but no Asteraceae and is the only site with *Verbena*. Durango has the highest precipitation in the first season (sampling period 1 and 2) and this is the site with more Asteraceae but no *Verbena*. Therefore, the apparent influence of site and precipitation on selectivity of the birds are probably due to differences in the seeds that are available in the soil seed bank at different sites.

Panicoideae

Panicoideae were the most common seeds in almost all of the bird species, site and sampling period combinations (Table 11), representing between 10 and 84 % of the diet of Baird’s and Grasshopper Sparrows with an average of 53 ± 19 % (Appendix B). Panicoideae in the study sites include *Panicum* spp., *Setaria* spp., *Botriochloa* spp., *Eriochloa* spp., and *Hackelochloa* spp. Soil samples indicate that *Panicum* spp. were the most common Panicoideae in Santa Teresa, *Eriochloa* spp. and *Panicum* spp. in Teseachi and *Panicum* spp. and *Setaria* spp. in El Uno. The variability in the amount of Panicoideae was not influenced by the availability of other preferred or abundant seeds (all P > 0.05 for *Pleuraphis* spp., Eragrostideae, *Verbena* spp., *Bouteloua* spp., and *Aristida* spp.). The amount of Panicoideae was neither influenced by the abundance of Panicoideae (F = 2.71, P = 0.175). Although the abundance of Panicoideae varied greatly between sites (Table 12), it is possible that birds were not limited in their consumption of Panicoideae seeds. The only variable significantly affecting the amount of Panicoideae in the diet was site (F = 9.03, P = 0.002). Thus, there must be some other difference between sites than seed availability that causes variation in the consumption of Panicoideae. There could be differences in vegetation characteristics such as associations between Panicoideae and other species, protection from predators around Panicoideae, and vegetation height and density that influence the amount of Panicoideae consumed.

Grass *vs*. Forb Seeds

In contrast to other studies in sparrow diets where forb and annual grass seeds were most important (Pulliam, 1986; Desmond *et al*., 2008), perennial grass seeds formed a large part of Baird's and Grasshopper Sparrow diets in the present study. Desmond *et al*. (2008) recognize that the dominance of annual grasses and forbs in their results might be due to the disturbed nature of their study sites. Here, the study sites all consisted of good quality (semi-) open grasslands dominated by native grasses, mainly perennial *Bouteloua* spp. and annual as well as perennial *Aristida* spp. However, *Aristida* spp. were hardly consumed. In this regard, Desmond *et al.* (2008) found that *Aristida* spp. seeds were only important in sparrow diets in late winter after seed abundance had declined substantially. The present study only investigated diets in early and mid-winter and it is possible that Baird's and Grasshopper Sparrows include more *Aristida* spp. in their diet towards the end of the winter. It has been suggested that sparrows would prefer forbs over grass seeds because they are unarmored (Pulliam, 1986), although Marone *et al*. (2008) found that sparrows of the Monte Desert in Argentina preferred grass over forb seeds. *Aristida* spp. have especially large awns which could explain why they are avoided. Although forbs did not form a large part of Baird's and Grasshopper Sparrow diets here, the forb *Verbena* spp. was preferred when present and the forb *Amaranthus* spp. was consumed in proportion to its availability. Possibly, Baird's and Grasshopper Sparrows did not consume more forb seeds because of their low availability in comparison to grass seeds. *Verbena* spp. were abundant in the soil seed bank at one of the study sites of Desmond *et al*. (2008) but here they were never detected in sparrow diets. However, Baird's and Grasshopper Sparrows were not present at these sites and different bird species may have different preferences (Pulliam, 1985; Desmond *et al*., 2008). Desmond *et al*. (2008) found that *Sporobolus* spp. seeds were preferred or at least important in the diets of all sparrows in their study, and they suggest that management practices should be favorable to promote the production of *Sporobolus* spp. seeds. Here, Baird's and Grasshopper Sparrow diets did not include *Sporobolus* spp. seeds. *Sporobolus* spp. seeds were never detected in the soil seed bank which explains their absence in Baird's and Grasshopper Sparrow diets. However, this shows that at least Baird's and Grasshopper Sparrows do not need *Sporobolus* spp. seeds to survive the winter.

Rather, management practices should promote seed production of Panicoideae and *Bouteloua* spp. seeds to ensure sufficient seed resources for over-wintering Baird’s and Grasshopper Sparrows.

CONCLUSIONS AND RECOMMENDATIONS

Baird’s and Grasshopper Sparrows did not consume seeds in accordance with their availability but showed preferences for certain seeds while others were avoided. Although their diets consisted of a large diversity of seeds, only a limited number of seeds was dominant in their diet. These seeds belonged to Panicoideae, *Bouteloua* spp., *Pleuraphis* spp., Eragrostideae, Asteraceae, *Verbena* spp. and *Amaranthus* spp., depending on their availability. Of these seeds, Panicoideae, *Pleuraphis* spp., Eragrostideae and *Verbena* spp. were consumed more than expected based on their availability in the soil seed bank, indicating preferences for these seeds. Although *Bouteloua* spp. seeds were not preferred, they formed a large part of the diets. This might be related to favorable vegetation characteristics associated with *Bouteloua* spp. The dominance of Panicoideae and *Bouteloua* spp. in the diets indicates that these seeds are important for the survival of overwintering Baird’s and Grasshopper Sparrows. It is therefore recommended to adopt grassland management practices that favor seed production of these two grasses, for example by reducing grazing pressure during the growing season. In general, (semi-) open grasslands where these grasses grow should be conserved. Further research is necessary to determine exactly how management practices such as grazing pressure and timing of grazing affect seed production of preferred and dominant seeds in the diet, and how this affects Baird’s and Grasshopper Sparrow survival. It would furthermore be interesting to study the diet composition of these two birds in sites where Panicoideae are relatively absent and in sites with a higher degree of disturbance to gain more information on dietary flexibility in compromised situations. Finally, it is recommended that future molecular diet studies test other barcode regions with a higher resolution, such that seeds can be identified at the species level.

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GENERAL DISCUSSION

The three studies showed that birds are selective in their diet. In captivity, Baird's and Grasshopper Sparrows differed in selectivity with Grasshopper Sparrow, the species with the larger bill, selecting larger seeds. In the field, there was no difference between the diets of the two bird species. In general, seed size appeared to be the main seed characteristic influencing selectivity in captivity. This is in agreement with previous research (Díaz, 1996). Seed selection based on size was related to handling efficiency. Seeds that are handled most efficiently are those seeds that maximize energy intake over time. Optimal foraging theory states that an animal will select those food items that maximize energy intake over time in relation to handling and searching time (Charnov, 1976). Grassland birds might therefore be foraging optimally.

In nature, birds included seeds from different sizes in their diet, although the relatively large Panicoideae seeds represented the largest part of the diets across different sites and sampling periods. Panicoideae here included *Panicum* spp., *Setaria* spp., *Botriochloa* spp., *Eriochloa* spp., and *Hackelochloa* spp. Second most common were *Bouteloua* spp. seeds. *Bouteloua* spp. included both larger seeds (*B. curtipendula*) and smaller seeds (mainly *B. gracilis*, *B. hirsuta* and *B. eriopoda*). Unfortunately it was not possible to discriminate between species due to the low resolution of the genetic barcode, the P6 loop of the *trnL* intron, used to identify the seeds. Possibly, seed size was also an important determinant of seed selection in the field, leading to preferences for the relatively large Panicoideae seeds. However, in nature handling efficiency also includes searching time which could explain why birds consume a more variable range of seed sizes in the field.

One common problem in (semi-) open grasslands of the Chihuahuan Desert, especially in communally owned grasslands (*ejidos*), is overgrazing (Desmond, 2004). Overgrazing has a negative impact on seed production. Overgrazing may furthermore lead to shrub encroachment, which limits grass cover and grass seed production even more (Eldridge *et al*., 2011). Climate change may worsen these effects and favor the spread of invasive species (Smith *et al*., 2000). The present work showed the dependence of Baird's and Grasshopper Sparrows on native grass seeds, in particular Panicoideae and *Bouteloua* spp. In captivity, only seeds from the introduced natal grass (*Melinis repens*) could be consumed, but seeds from the introduced Lehmann lovegrass (*Eragrostis lehmanniana*) and buffelgrass (*Pennisetum ciliare*) were avoided in captivity. Based on these results it can be predicted that overgrazing, shrub encroachment, and climate change will negatively affect habitat suitability for Baird's and Grasshopper Sparrows.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Baird’s and Grasshopper Sparrows are selective in their diet. Both species depend on native grasses, especially Panicoideae and *Bouteloua* spp. Therefore, (semi-) open, native grasslands should be conserved. Management practices should favor seed production of Panicoideae and *Bouteloua* spp. in particular, and native grasses in general, and actively prevent and control the spread of invasive species and shrub encroachment. Further research is necessary to determine exactly how management practices such as grazing pressure and timing of grazing affect seed production of common grasses in the diets, and how this affects Baird’s and Grasshopper Sparrow populations. It would also be interesting to investigate Baird’s and Grasshopper Sparrow diets in more disturbed sites, including areas invaded by natal grass, to see whether these birds also consume large amounts of natal grass seeds in the field. Finally, it is recommended that diet studies in the future investigate the use of other molecular markers, to be able to identify seeds to the species level.

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APPENDIX A: BOTANICAL COMPOSITION OF THE STUDY SITES COMPARED WITH THE SOIL SEED BANK

For seeds in the soil seed bank that were not encountered in the vegetation transects only those that had an abundance of 5 % or more are reported.

Table 1. Botanical composition and soil seed bank at Santa Teresa, Durango, Mexico

Species	Dominance (%)	Soil Nov 2012 (%)	Soil Jan 2013 (%)	Soil Jan 2014 (%)
Aristida spp. (annual)	3.8	6.22	7.29	16.55
Aristida spp. (perennial)	0.67			
Asteraceae	0.56	20.51	27.49	46.68
Baccharis pteronioides	0.22	0	0	0
Botriochloa barbinodis	0.11	0	0	0
Bouteloua curtipendula	4.26	0	0	0.98
B. gracilis	34.19	13.36	17.23	3.09
B. hirsuta	0.11	0	0	0
Brickellia spinulosa	2.24	0	0	0
Buddleia scordioides	0.11	0	0	0
Chloris virgata	0.56	10.93	10.64	8.51
Condalia ericoides	1.01	0	0	0
Dichondria argentea	1.01	0	0	0
Dyschoriste schiedeana	0.34	0	0	0
Dyssodia papposa	0.11	0.91	0.03	0
Enneapogon desvauxii	7.62	6.71	9.42	0.22
Ephedra trifurca	0.45	0	0	0
Eragrostis cilianensis	0	0.14	0.76	6.78
Eupatorium odoratum	0.11	0	0	0
Eysenhardtia spinosa	1.01	0	0	0
E. texana	0.11	0	0	0
Juniperus monosperma	3.25	0	0	0
Mimosa biuncifera	0.11	0	0	0
Muhlenbergia phleoides	0.11	0	0	0.01
M. rigida	0.22	0	0.08	0
Panicum hallii	2.13	3.43	1.88	0.80
P. obtusum	0.11	0.19	0	0
Pectis papossa	2.45	0	0	0
Pleuraphis mutica	31.61	2.79	3.30	0.39
Prosopis glandulosa	0.11	0	0	0
Rhus microphya	0.45	0	0	0
Triquilia canescens	0.11	0	0	0
Zinnia grandiflora	0.11	0	0	0
Unidentified 1	0.11			
Unidentified 2	0.11			
Unidentified 3	0.11			

Table 2. Botanical composition and soil seed bank at Teseachi, Chihuahua, Mexico

Species	Dominance (%)	Soil Nov 2012 (%)	Soil Jan 2013 (%)	Soil Jan 2014 (%)
Aristida spp.	8.04	7.47	5.79	52.97
Botriochloa barbinoides	0.10	0.44	0.08	0.12
Bouteloua curtipendula	0.19	0	0	0
B. gracilis	75.48	3.44	2.23	1.29
B. hirsuta	4.26	4.14	3.97	3.44
Chloris virgata	0	3.05	6.86	7.25
Commelina dianthifolia	0.10	0	0	0
Cyperus spp.	1.07	0	0	0
Elyonurus barbiculmis	0.10	0	0	0
Eragrostis spp.	0	2.67	7.56	10.87
Eriochloa spp.	0	6.37	19.77	5.27
Hymenoxys odorata	0	34.62	1.13	0
Lycurus phleoides	1.94	10.28	3.68	2.98
Muhlenbergia minutissima	0.97	0	0	0
M. rigida	3.78	0	0	0
Muhlenbergia spp.	0.19	0.84	0.24	0.02
Panicum bulbosum	0	0.46	1.00	6.60
Plantago patagonica	0.29	0	0	0
Polygonum aveniculare	0	8.07	16.41	1.39
Schizachyrium cirratum	2.81	0	0	0
Trachypogon secundus	0.10	0	0	0
Verbena neomexicana	0.39	3.24	9.37	0.65
Unidentified	0.20			

Table 3. Botanial compostion and soil seed bank at El Uno – Centro, Chihuahua, Mexico

Species	Dominance (%)	Soil Nov 2012 (%)	Soil Jan 2013 (%)	Soil Jan 2014 (%)
Acacia angustissima	0.21	0	0	0
Aristida adscencionis	4.83	4.40	12.66	3.78
A. orcutiana	1.05	0	0	0
Aristida spp. (annual)	8.19	0	0	0
Aristida spp. (perennial)	22.27	0	0	0
Atriplex tuberculata	0	70.48	46.86	1.30
Botriochloa barbinoides	1.26	0.44	0.98	2.18
Bouteloua barbata	0.63	1.07	0.01	3.55
B. eriopoda	24.37	0.22	2.07	0.63
B. gracilis	6.09	1.06	1.13	0
B. hirsuta	13.45	2.18	2.97	0.48
Ephedra trifurca	11.55	0	0	0
Eragrostis cilianensis	0	0	0	6.54
Evolvulus alsinoides	0.21	0	0	0
Haplopappus gracilis	0	4.02	6.93	2.15
Hofmansegia glauca	0.63	0	0	0
Machaeranthera pinnatifida	0.42	1.63	5.19	0.02
Mollugo verticillata	0	0	0	66.74
Panicum bulbosum	2.10	0.38	2.33	0
P. hirsutum	0.21	0.34	0.17	2.48
Pleuraphis mutica	0.21	0.22	0.08	0.54
Prosopis glandulosa	1.05	0	0	0
Salsola kali	0.42	0.57	2.12	2.04
Sida procumbens	0.21	0.01	0	0
Solanum elaeagnifolium	0.21	0	0	0
Zinnia acerosa	0.42	0	0	0

Table 4. Botanical composition and soil seed bank at El Uno – Los Ratones, Chihuahua, Mexico

Species	Dominance (%)	Soil Jan 2014 (%)
Acacia angustissima	0.33	0
Amaranthus spp.	0.33	0
A. palmeri	0.50	35.20
Apodanthera undulata	0.33	0
Aristida adscensionis	40.13	4.79
A. longiseta	0.33	0
A. orcuttiana	0.84	0
Aristida spp. (perennial)	4.52	0
Botriochloa barbinodis	0.17	0.05
Bouteloua aristidoides	1.34	2.84
B. barbata	0.17	3.05
B. eriopoda	5.85	3.41
B. gracilis	9.03	0.02
B. hirsuta	0.33	0
Chloris virgata	0.17	0.53
Croton pottsii	0.33	0
Enneapogon desvauxii	0.17	0
Ephedra trifurca	0.17	0
Eragrostis cilianensis	0	12.11
E. superba	0	5.33
Evolvulus alsinoides	0.17	0
E. nuttallianus	0.33	0
Gutierrezia sarothrae	0.17	0
Pleuraphis mutica	24.08	0
Krameria grayi	0.17	0
Leguminosa	0.17	
Machaeranthera pinnatifida	0.17	0
Mimosa biuncifera	0.17	0
Mollugo verticillata	0	8.09
Panicum obtusum	1.17	0.50
Panicum spp. (annual)	0.50	5.43
Prosopis glandulosa	3.51	0
Salsola iberica	2.17	0.31
Scleropogon brevifolius	0.67	0
Sida procumbens	0.33	0
Solanum elaeagnifolium	0.50	0
Sporobolus spp.	0.50	0
Unidentified	0.17	

APPENDIX B. DNA BARCODING RESULTS

DNA Barcoding results are shown for every pool grouped per genus. As mentioned in the main text, the resolution of the genetic barcode (P6 loop of the trnL intron (Taberlet *et al*., 2007)) was too low to discriminate reliably between species. In some cases the results for genus were also questionable in which case seeds were grouped into families for further analysis (see main text).

Table 5. DNA barcoding results for pool 1: Baird's Sparrow (*Ammodramus bairdii*), Santa Teresa, Durango, Mexico, November 2012

Genus	No. of reads	% of total reads	% of mapped reads
Botriochloa	488682	13.83	15.13
Panicum	430718	12.19	13.33
Setaria	314655	8.90	9.74
Hackelochloa	311347	8.81	9.64
Eriochloa	281978	7.98	8.73
Pleuraphis	225759	6.39	6.99
Bouteloua	187583	5.31	5.81
Muhlenbergia	179295	5.07	5.55
Lycurus	178832	5.06	5.54
Hypochaeris	171408	4.85	5.31
Verbena	65023	1.84	2.01
Verbesina	57449	1.63	1.78
Parthenium	57446	1.63	1.78
Helenium	57359	1.62	1.78
Amaranthus	53962	1.53	1.67
Eragrostis	48994	1.39	1.52
Enneapogon	44747	1.27	1.39
Bromus	24745	0.70	0.77
Machaeranthera	11174	0.32	0.35
Bidens	8331	0.24	0.26
Senecio	7679	0.22	0.24
Euphorbia	6581	0.19	0.20
Megathyrsus	4575	0.13	0.14
Gaura	4118	0.12	0.13
Chloris	1702	0.05	0.05
Datura	1486	0.04	0.05
Achillea	1123	0.03	0.03
Mollugo	957	0.03	0.03
Chenopodium	789	0.02	0.02
Descurainia	452	0.01	0.01
Portulaca	436	0.01	0.01
Solanum	143		
Dyssodia	143		
Elymus	138		
Allium	80		
Salsola	71		
Brassica	47		
Lamium	29		
Aristida	25		
Cucurbita	17		
Schkuria	11		
Plantago	6		
Thlaspi	6		
Sporobolus	5		
Polygonum	2		
Digitaria	2		
Read sum mapped	3230110		
Read sum total	3534032		

Table 6. DNA barcoding results for pool 2: Grasshopper Sparrow (*Ammodramus savannarum*), Santa Teresa, Durango, Mexico November 2012

Genus	No. of reads	% of total reads	% of mapped reads
Bouteloua	101068	31.93	35.01
Hypochaeris	54087	17.09	18.73
Pleuraphis	23336	7.37	8.08
Parthenium	18175	5.74	6.30
Verbesina	18065	5.71	6.26
Helenium	18058	5.71	6.25
Panicum	11191	3.54	3.88
Euphorbia	10828	3.42	3.75
Eriochloa	10794	3.41	3.74
Setaria	4985	1.58	1.73
Hackelochloa	4952	1.56	1.72
Lycurus	4094	1.29	1.42
Muhlenbergia	4010	1.27	1.39
Senecio	1511	0.48	0.52
Botriochloa	1255	0.40	0.43
Bidens	510	0.16	0.18
Achillea	458	0.14	0.16
Eragrostis	329	0.10	0.11
Dyssodia	233	0.07	0.08
Solanum	216	0.07	0.07
Bromus	192	0.06	0.07
Chloris	109	0.03	0.04
Enneapogon	96	0.03	0.03
Megathyrsus	93	0.03	0.03
Datura	28	0.01	0.01
Verbena	19	0.01	0.01
Elymus	7		
Machaeranthera	5		
Schkuria	2		
Allium	2		
Aristida	1		
Hymenoxys	1		
Portulaca	1		
Read sum mapped	288711		
Read sum total	316490		

Table 7. DNA barcoding results for pool 3: Baird’s Sparrow (*Ammodramus bairdii*), Teseachi, Chihuahua, Mexico, November 2012

Genus	No. of reads	% of total reads	% of mapped reads
Bouteloua	88757	29.16	31.92
Panicum	34588	11.36	12.44
Eriochloa	26921	8.85	9.68
Setaria	26517	8.71	9.54
Pleuraphis	16704	5.49	6.01
Muhlenbergia	15756	5.18	5.67
Lycurus	15599	5.13	5.61
Eragrostis	1206	0.40	0.43
Bromus	581	0.19	0.21
Megathyrsus	152	0.05	0.05
Chloris	141	0.05	0.05
Hypochaeris	135	0.04	0.05
Verbena	124	0.04	0.04
Euphorbia	115	0.04	0.04
Enneapogon	105	0.03	0.04
Parthenium	43	0.01	0.02
Helenium	43	0.01	0.02
Verbesina	37	0.01	0.01
Bidens	17	0.01	0.01
Brassica	15		0.01
Datura	10		
Senecio	8		
Machaeranthera	2		
Allium	2		
Cucurbita	1		
Digitaria	1		
Read sum mapped	278092		
Read sum total	304364		

Table 8. DNA barcoding results for pool 4: Grasshopper Sparrow (*Ammodramus savannarum*), Teseachi, Chihuahua, Mexico, November 2012

Genus	No. of reads	% of total reads	% of mapped reads
Bouteloua	19144	19.09	22.50
Eriochloa	16980	16.93	19.96
Panicum	11628	11.60	13.67
Setaria	8768	8.74	10.31
Hackelochloa	8188	8.17	9.63
Pleuraphis	4582	4.57	5.39
Botriochloa	4155	4.14	4.88
Muhlenbergia	3604	3.59	4.24
Lycurus	3487	3.48	4.10
Hypochaeris	1289	1.29	1.52
Eragrostis	532	0.53	0.63
Euphorbia	414	0.41	0.49
Verbesina	403	0.40	0.47
Parthenium	389	0.39	0.46
Helenium	384	0.38	0.45
Bromus	269	0.27	0.32
Megathyrsus	230	0.23	0.27
Enneapogon	205	0.20	0.24
Verbena	144	0.14	0.17
Senecio	99	0.10	0.12
Chloris	35	0.03	0.04
Bidens	24	0.02	0.03
Datura	23	0.02	0.03
Thlaspi	20	0.02	0.02
Brassica	18	0.02	0.02
Machaeranthera	12	0.01	0.01
Allium	10	0.01	0.01
Elymus	8	0.01	0.01
Cucurbita	7	0.01	0.01
Achillea	6	0.01	0.01
Dyssodia	3		
Amaranthus	2		
Aristida	2		
Descurainia	1		
Portulaca	1		
Read sum mapped	85066		
Read sum total	100273		

Table 9. DNA barcoding results for pool 5: Baird’s Sparrow (*Ammodramus bairdii*), El Uno - Centro, Chihuahua, Mexico, November 2012

Genus	No. of reads	% of total reads	% of mapped reads
Panicum	66942	25.46	27.81
Botriochloa	51076	19.43	21.22
Hackelochloa	50494	19.21	20.98
Setaria	50329	19.14	20.91
Bouteloua	17034	6.48	7.08
Machaeranthera	1589	0.60	0.66
Eriochloa	878	0.33	0.36
Pleuraphis	399	0.15	0.17
Muhlenbergia	290	0.11	0.12
Lycurus	257	0.10	0.11
Eragrostis	251	0.10	0.10
Chloris	187	0.07	0.08
Hypochaeris	168	0.06	0.07
Enneapogon	131	0.05	0.05
Bromus	106	0.04	0.04
Gaura	105	0.04	0.04
Parthenium	68	0.03	0.03
Verbesina	53	0.02	0.02
Elymus	51	0.02	0.02
Helenium	50	0.02	0.02
Chenopodium	50	0.02	0.02
Megathyrsus	45	0.02	0.02
Amaranthus	39	0.01	0.02
Solanum	26	0.01	0.01
Aristida	17	0.01	0.01
Senecio	12		
Bidens	11		
Euphorbia	9		
Verbena	9		
Datura	5		
Lamium	2		
Cucurbita	2		
Dyssodia	1		
Achillea	1		
Read sum mapped	240687		
Read sum total	262910		

Table 10. DNA barcoding results for pool 6: Grasshopper Sparrow (*Ammodramus savannarum*), El Uno - Centro, Chihuahua, Mexico, November 2012

Genus	No. of reads	% of total reads	% of mapped reads
Panicum	54241	24.32	27.34
Hackelochloa	40866	18.32	20.60
Setaria	40754	18.27	20.54
Botriochloa	19016	8.53	9.58
Pleuraphis	12883	5.78	6.49
Lycurus	12732	5.71	6.42
Muhlenbergia	12674	5.68	6.39
Machaeranthera	2066	0.93	1.04
Eriochloa	1026	0.46	0.52
Chloris	278	0.12	0.14
Bouteloua	266	0.12	0.13
Chenopodium	238	0.11	0.12
Hypochaeris	218	0.10	0.11
Elymus	203	0.09	0.10
Eragrostis	192	0.09	0.10
Euphorbia	150	0.07	0.08
Enneapogon	112	0.05	0.06
Bromus	87	0.04	0.04
Allium	78	0.03	0.04
Helenium	74	0.03	0.04
Parthenium	68	0.03	0.03
Verbesina	62	0.03	0.03
Megathyrsus	22	0.01	0.01
Salsola	20	0.01	0.01
Descurainia	17	0.01	0.01
Aristida	11		0.01
Senecio	10		0.01
Bidens	7		
Amaranthus	7		
Verbena	6		
Datura	4		
Thlaspi	3		
Brassica	1		
Mollugo	1		
Sporobolus	1		
Read sum mapped	198394		
Read sum total	223029		

Table 11. DNA barcoding results for pool 7: Baird's Sparrow (*Ammodramus bairdii*), Santa Teresa, Durango, Mexico, January 2013

Genus	No. of reads	% of total reads	% of mapped reads
Pleuraphis	118152	30.30	32.35
Botriochloa	49292	12.64	13.50
Panicum	36830	9.44	10.09
Eriochloa	35525	9.11	9.73
Setaria	26350	6.76	7.22
Hackelochloa	26271	6.74	7.19
Bouteloua	22735	5.83	6.23
Enneapogon	10957	2.81	3.00
Lycurus	10693	2.74	2.93
Muhlenbergia	10544	2.70	2.89
Hypochaeris	6139	1.57	1.68
Helenium	2139	0.55	0.59
Parthenium	2108	0.54	0.58
Verbesina	2033	0.52	0.56
Eragrostis	1237	0.32	0.34
Euphorbia	925	0.24	0.25
Megathyrsus	875	0.22	0.24
Bromus	566	0.15	0.15
Verbena	552	0.14	0.15
Chloris	289	0.07	0.08
Gaura	247	0.06	0.07
Elymus	201	0.05	0.06
Senecio	146	0.04	0.04
Bidens	76	0.02	0.02
Descurainia	61	0.02	0.02
Allium	60	0.02	0.02
Thlaspi	55	0.01	0.02
Salsola	43	0.01	0.01
Brassica	32	0.01	0.01
Datura	16		
Dyssodia	12		
Achillea	9		
Schkuria	5		
Solanum	4		
Amaranthus	3		
Chenopodium	1		
Machaeranthera	1		
Cucurbita	1		
Read sum mapped	365185		
Read sum total	389963		

Table 12. DNA barcoding results for pool 8: Grasshopper Sparrow (*Ammodramus savannarum*), Santa Teresa, Durango, Mexico, January 2013

Genus	No. of reads	% of total reads	% of mapped reads
Bouteloua	146057	34.69	37.15
Eriochloa	47938	11.39	12.19
Pleuraphis	37609	8.93	9.57
Panicum	34454	8.18	8.76
Setaria	24728	5.87	6.29
Hackelochloa	24538	5.83	6.24
Botriochloa	16175	3.84	4.11
Muhlenbergia	15439	3.67	3.93
Lycurus	15177	3.60	3.86
Datura	14133	3.36	3.59
Enneapogon	9487	2.25	2.41
Hypochaeris	1946	0.46	0.49
Euphorbia	898	0.21	0.23
Megathyrsus	780	0.19	0.20
Helenium	696	0.17	0.18
Parthenium	633	0.15	0.16
Verbesina	622	0.15	0.16
Eragrostis	588	0.14	0.15
Bromus	426	0.10	0.11
Verbena	354	0.08	0.09
Chloris	206	0.05	0.05
Senecio	95	0.02	0.02
Allium	41	0.01	0.01
Elymus	33	0.01	0.01
Machaeranthera	22	0.01	0.01
Gaura	20		0.01
Portulaca	18		
Thlaspi	10		
Bidens	9		
Solanum	9		
Achillea	2		
Cucurbita	1		
Aristida	1		
Dyssodia	1		
Read sum mapped	393146		
Read sum total	421051		

Table 13. DNA barcoding results for pool 9: Baird's Sparrow (*Ammodramus bairdii*), Teseachi, Chihuahua, Mexico, January 2013

Genus	No. of reads	% of total reads	% of mapped reads
Verbena	94815	23.34	24.76
Panicum	52380	12.89	13.68
Hackelochloa	39036	9.61	10.20
Setaria	39010	9.60	10.19
Hackelochloa	39036	9.61	10.20
Hypochaeris	28984	7.13	7.57
Eriochloa	26556	6.54	6.94
Pleuraphis	15804	3.89	4.13
Muhlenbergia	14255	3.51	3.72
Lycurus	14202	3.50	3.71
Bouteloua	13949	3.43	3.64
Botriochloa	10347	2.55	2.70
Helenium	9705	2.39	2.53
Verbesina	9657	2.38	2.52
Parthenium	9503	2.34	2.48
Senecio	1502	0.37	0.39
Bidens	1383	0.34	0.36
Eragrostis	806	0.20	0.21
Bromus	423	0.10	0.11
Megathyrsus	145	0.04	0.04
Enneapogon	123	0.03	0.03
Euphorbia	83	0.02	0.02
Achillea	66	0.02	0.02
Allium	59	0.01	0.02
Chloris	22	0.01	0.01
Dyssodia	19		
Datura	19		
Solanum	9		
Thlaspi	4		
Brassica	4		
Elymus	2		
Machaeranthera	2		
Descurainia	1		
Schkuria	1		
Hymenoxys	1		
Read sum mapped	382877		
Read sum total	406244		

Table 14. DNA barcoding results for pool 10: Grasshopper Sparrow (*Ammodramus savannarum*), Teseachi, Chihuahua, Mexico, January 2013

Genus	No. of reads	% of total reads	% of mapped reads
Eriochloa	115819	27.02	29.04
Verbena	53122	12.39	13.32
Hypochaeris	52650	12.28	13.20
Panicum	20516	4.79	5.14
Helenium	17641	4.12	4.42
Verbesina	17476	4.08	4.38
Parthenium	17468	4.07	4.38
Bouteloua	14029	3.27	3.52
Botriochloa	13848	3.23	3.47
Eragrostis	13117	3.06	3.29
Hackelochloa	12899	3.01	3.23
Setaria	12781	2.98	3.20
Pleuraphis	10804	2.52	2.71
Lycurus	7556	1.76	1.89
Muhlenbergia	7431	1.73	1.86
Bromus	6668	1.56	1.67
Senecio	2895	0.68	0.73
Megathyrsus	1533	0.36	0.38
Bidens	167	0.04	0.04
Achillea	153	0.04	0.04
Enneapogon	93	0.02	0.02
Chloris	88	0.02	0.02
Dyssodia	27	0.01	0.01
Euphorbia	16		
Elymus	9		
Allium	8		
Datura	7		
Schkuria	4		
Brassica	4		
Cucurbita	4		
Machaeranthera	3		
Aristida	1		
Read sum mapped	398837		
Read sum total	428691		

Table 15. DNA barcoding results for pool 11: Baird’s Sparrow (*Ammodramus bairdii*), El Uno - Centro, Chihuahua, Mexico, January 2013

Genus	No. of reads	% of total reads	% of mapped reads
Botriochloa	147307	34.65	37.44
Panicum	65826	15.48	16.73
Setaria	49437	11.63	12.57
Hackelochloa	49354	11.61	12.54
Pleuraphis	21919	5.16	5.57
Muhlenbergia	21520	5.06	5.47
Lycurus	21488	5.05	5.46
Machaeranthera	6866	1.61	1.75
Eriochloa	4354	1.02	1.11
Datura	1419	0.33	0.36
Chenopodium	771	0.18	0.20
Hypochaeris	652	0.15	0.17
Bouteloua	461	0.11	0.12
Eragrostis	302	0.07	0.08
Parthenium	235	0.06	0.06
Verbesina	234	0.06	0.06
Helenium	234	0.06	0.06
Euphorbia	168	0.04	0.04
Bromus	160	0.04	0.04
Chloris	149	0.04	0.04
Verbena	138	0.03	0.04
Megathyrsus	106	0.02	0.03
Enneapogon	101	0.02	0.03
Gaura	54	0.01	0.01
Descurainia	45	0.01	0.01
Senecio	25	0.01	0.01
Bidens	23	0.01	0.01
Brassica	21		0.01
Allium	14		
Elymus	12		
Aristida	10		
Thlaspi	6		
Salsola	4		
Amaranthus	4		
Achillea	4		
Solanum	3		
Sporobolus	2		
Cucurbita	1		
Mollugo	1		
Dyssodia	1		
Read sum mapped	393431		
Read sum total	425142		

Table 16. DNA barcoding results for pool 12: Grasshopper Sparrow (*Ammodramus savannarum*), El Uno - Centro, Chihuahua, Mexico, January 2013

Genus	No. of reads	% of total reads	% of mapped reads
Botriochloa	84870	21.95	23.86
Panicum	45242	11.70	12.72
Pleuraphis	39062	10.10	10.98
Muhlenbergia	38422	9.94	10.80
Lycurus	38120	9.86	10.72
Setaria	33689	8.71	9.47
Amaranthus	3943	1.02	1.11
Hypochaeris	2995	0.77	0.84
Bouteloua	2703	0.70	0.76
Euphorbia	1103	0.29	0.31
Verbesina	1057	0.27	0.30
Helenium	1055	0.27	0.30
Parthenium	1052	0.27	0.30
Eragrostis	842	0.22	0.24
Megathyrsus	541	0.14	0.15
Enneapogon	462	0.12	0.13
Bromus	391	0.10	0.11
Machaeranthera	339	0.09	0.10
Verbena	291	0.08	0.08
Chloris	187	0.05	0.05
Senecio	156	0.04	0.04
Salsola	49	0.01	0.01
Datura	25	0.01	0.01
Elymus	24	0.01	0.01
Lamium	22	0.01	0.01
Brassica	19		0.01
Bidens	9		
Cucurbita	9		
Aristida	8		
Chenopodium	6		
Achillea	5		
Dyssodia	4		
Allium	4		
Solanum	2		
Sporobolus	1		
Read sum mapped	355715		
Read sum total	386686		

Table 17. DNA barcoding results for pool 13: Baird's Sparrow (*Ammodramus bairdii*), Santa Teresa, Durango, Mexico, January 2014

Genus	No. of reads	% of total reads	% of mapped reads
Hypochaeris	81537	18.21	19.78
Pleuraphis	59620	13.31	14.46
Muhlenbergia	47288	10.56	11.47
Lycurus	47186	10.54	11.45
Enneapogon	28866	6.45	7.00
Verbesina	27163	6.07	6.59
Helenium	27143	6.06	6.58
Parthenium	27022	6.03	6.56
Eragrostis	17581	3.93	4.27
Bromus	8848	1.98	2.15
Bidens	7205	1.61	1.75
Eriochloa	6461	1.44	1.57
Panicum	5460	1.22	1.32
Bouteloua	4846	1.08	1.18
Gaura	4053	0.90	0.98
Senecio	3635	0.81	0.88
Hackelochloa	2455	0.55	0.60
Setaria	2402	0.54	0.58
Machaeranthera	1766	0.39	0.43
Achillea	731	0.16	0.18
Chloris	596	0.13	0.14
Botriochloa	163	0.04	0.04
Megathyrsus	61	0.01	0.01
Euphorbia	47	0.01	0.01
Dyssodia	43	0.01	0.01
Amaranthus	12		
Verbena	4		
Lamium	4		
Allium	3		
Schkuria	2		
Aristida	2		
Elymus	1		
Chenopodium	1		
Brassica	1		
Portulaca	1		
Read sum mapped	412209		
Read sum total	447858		

Table 18. DNA barcoding results for pool 14: Baird's Sparrow (*Ammodramus bairdii*), Teseachi, Chihuahua, Mexico, January 2014

Genus	No. of reads	% of total reads	% of mapped reads
Botriochloa	120833	28.21	32.73
Bouteloua	55802	13.03	15.12
Pleuraphis	26306	6.14	7.13
Panicum	26049	6.08	7.06
Muhlenbergia	25258	5.90	6.84
Lycurus	25084	5.86	6.80
Eriochloa	22749	5.31	6.16
Hackelochloa	18616	4.35	5.04
Setaria	18599	4.34	5.04
Eragrostis	13463	3.14	3.65
Verbena	7564	1.77	2.05
Bromus	6857	1.60	1.86
Megathyrsus	712	0.17	0.19
Hypochaeris	536	0.13	0.15
Parthenium	191	0.04	0.05
Verbesina	188	0.04	0.05
Helenium	185	0.04	0.05
Chloris	46	0.01	0.01
Senecio	31	0.01	0.01
Bidens	24	0.01	0.01
Enneapogon	22	0.01	0.01
Amaranthus	8		
Achillea	8		
Datura	3		
Gaura	3		
Digitaria	2		
Salsola	1		
Polygonum	1		
Euphorbia	1		
Cucurbita	1		
Dyssodia	1		
Elymus	1		
Read sum mapped	369145		
Read sum total	428304		

Table 19. DNA barcoding results for pool 15: Grasshopper Sparrow (*Ammodramus savannarum*), Teseachi, Chihuahua, Mexico, January 2014

Genus	No. of reads	% of total reads	% of mapped reads
Botriochloa	97495	22.76	24.90
Eriochloa	56701	13.24	14.48
Panicum	53979	12.60	13.78
Bouteloua	42466	9.91	10.84
Setaria	38314	8.94	9.78
Hackelochloa	38260	8.93	9.77
Pleuraphis	20345	4.75	5.20
Muhlenbergia	18675	4.36	4.77
Lycurus	18490	4.32	4.72
Verbena	2597	0.61	0.66
Megathyrsus	1236	0.29	0.32
Machaeranthera	957	0.22	0.24
Eragrostis	811	0.19	0.21
Bromus	438	0.10	0.11
Hypochaeris	242	0.06	0.06
Verbesina	87	0.02	0.02
Helenium	75	0.02	0.02
Parthenium	74	0.02	0.02
Euphorbia	74	0.02	0.02
Elymus	74	0.02	0.02
Chloris	67	0.02	0.02
Enneapogon	54	0.01	0.01
Dyssodia	47	0.01	0.01
Bidens	14		
Senecio	9		
Amaranthus	9		
Datura	6		
Achillea	2		
Gaura	2		
Aristida	2		
Sporobolus	1		
Brassica	1		
Read sum mapped	391604		
Read sum total	428372		

Table 20. DNA barcoding results for pool 16: Grasshopper Sparrow (*Ammodramus savannarum*), El Uno – Centro, Chihuahua, Mexico, January 2014

Genus	No. of reads	% of total reads	% of mapped reads
Panicum	134300	31.44	34.21
Setaria	102103	23.90	26.01
Hackelochloa	100613	23.55	25.63
Botriochloa	19814	4.64	5.05
Pleuraphis	13320	3.12	3.39
Lycurus	7594	1.78	1.93
Muhlenbergia	7377	1.73	1.88
Eriochloa	1808	0.42	0.46
Eragrostis	1370	0.32	0.35
Mollugo	903	0.21	0.23
Bromus	725	0.17	0.18
Machaeranthera	663	0.16	0.17
Descurainia	407	0.10	0.10
Portulaca	362	0.08	0.09
Chloris	316	0.07	0.08
Hypochaeris	169	0.04	0.04
Bouteloua	136	0.03	0.03
Bidens	73	0.02	0.02
Helenium	72	0.02	0.02
Enneapogon	69	0.02	0.02
Verbesina	67	0.02	0.02
Megathyrsus	66	0.02	0.02
Parthenium	62	0.01	0.02
Verbena	39	0.01	0.01
Euphorbia	20		0.01
Salsola	16		
Elymus	14		
Senecio	14		
Datura	11		
Amaranthus	9		
Chenopodium	9		
Plantago	4		
Gaura	2		
Aristida	2		
Lamium	1		
Brassica	1		
Read sum mapped	392531		
Read sum total	427170		

Table 21. DNA barcoding results for pool 17: Grasshopper Sparrow (*Ammodramus savannarum*), El Uno – Los Ratones, Chihuahua, Mexico, January 2014

Genus	No. of reads	% of total reads	% of mapped reads
Panicum	70873	20.01	21.39
Setaria	53636	15.15	16.19
Hackelochloa	53351	15.07	16.10
Amaranthus	49975	14.11	15.08
Eriochloa	47410	13.39	14.31
Bouteloua	20896	5.90	6.31
Pleuraphis	12332	3.48	3.72
Muhlenbergia	8121	2.29	2.45
Lycurus	8058	2.28	2.43
Euphorbia	2822	0.80	0.85
Botriochloa	1313	0.37	0.40
Eragrostis	856	0.24	0.26
Machaeranthera	571	0.16	0.17
Bromus	420	0.12	0.13
Megathyrsus	178	0.05	0.05
Chloris	121	0.03	0.04
Hypochaeris	94	0.03	0.03
Enneapogon	77	0.02	0.02
Mollugo	53	0.01	0.02
Verbesina	40	0.01	0.01
Parthenium	35	0.01	0.01
Helenium	27	0.01	0.01
Bidens	13		
Verbena	9		
Datura	9		
Senecio	5		
Achillea	4		
Solanum	3		
Chenopodium	2		
Lamium	2		
Plantago	2		
Salsola	1		
Sporobolus	1		
Allium	1		
Polygonum	1		
Read sum mapped	331312		
Read sum total	354111		

APPENDIX C: BIRD MORPHOLOGICAL MEASUREMENTS

Table 22. Mean morphological measurements (± S.D.) of Baird's Sparrow (*Ammodramus bairdii*) and Grasshopper Sparrow (*A. savannarum*)

Species	n	Weight (g)	Fat (0-5)	Wing (mm)	Tail (mm)	Bill length (mm)	Bill width (mm)	Bill depth (mm)
Baird's Sparrow	158	18.2 (1.4)	1.3 (0.9)	67.9 (2.3)	51.9 (2.5)	10.1 (0.5)	5.6 (0.3)	5.6 (0.3)
Grasshopper Sparrow	188	17.3 (1.0)	1.3 (1.0)	61.2 (1.9)	47.9 (2.2)	10.7 (0.4)	5.8 (0.4)	5.9 (0.3)