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Seed Nutritional Traits and Bird Feeding Preferences: Laboratory Evaluation with an Exploratory Quantitative Literature Synthesis

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Abstract

Avian species impose considerable selective influences on plant ecosystems through seed consumption and endozoochorous seed dissemination; yet, the physiological and nutritional factors that dictate bird dietary preferences are not fully understood. According to the nutritional hypothesis, foraging avians are believed to weigh the metabolic dietary benefits against the costs of processing and detoxifying plant secondary compounds. In this study, we performed an empirical laboratory assessment of total soluble protein, free glucose, total phenolics, and physical handling resistance among 10 selected granivorous bird seed species utilising four separate biological replicates (n = 4). Laboratory analysis revealed significant interspecific variability: Brassica napus (rapeseed) and Carthamus tinctorius (safflower) displayed elevated soluble protein levels (16.61 ± 0.46 and 12.48 ± 1.00 mg/g dry weight, respectively) alongside considerable phenolic defence mechanisms (4.11 ± 0.17 and 1.84 ± 0.40 mg gallic acid equivalents [GAE]/g). In contrast, staple grass grains (Panicum miliaceum, Setaria italica, and Oryza sativa) demonstrated minimal phenolic content (< 0.10 mg GAE/g) and significant free glucose levels (up to 1.57 ± 0.81 µmol/g), leading to higher nutrient-to-defense ratios. Simultaneously, a quantitative literature synthesis was performed to contextualise these characteristics in relation to documented bird feeding preferences. Standardised impact estimates derived from existing literature indicated a positive association between seed protein levels and avian selection (r = 0.26, 95% CI: 0.02 to 0.48, p = 0.038) as determined by one qualifying study. The unmodified aggregation of six phenolic effect sizes indicated a robust dietary avoidance (pooled r = -0.79, 95% CI: -0.93 to -0.44, p < 0.001), yet the sensitivity analysis at the study level rendered this association nonsignificant (r = -0.60, 95% CI: -0.94 to 0.32, p = 0.188) owing to the dependence of effect sizes. The laboratory experiments analysed chemical and physical characteristics without incorporating live-bird preference bioassays; thus, our experimental results delineate the nutritional landscape of accessible diaspores but do not conclusively demonstrate eating preferences. These findings indicate that the chemical defences and physical toughness of seeds influence the availability of protein-rich diaspores, underscoring the need of assessing multi-trait foraging trade-offs in avian nutritional ecology.

INTRODUCTION

Granivorous and frugivorous avians serve as essential catalysts for terrestrial plant establishment, fulfilling two ecological functions as both harmful pre-dispersal seed consumers and beneficial seed dispersers [1, 2]. Avian selective feeding imposes significant evolutionary influences on diaspore characteristics, directly affecting seedling establishment trends, plant community demographics, and forest regeneration processes [2, 3]. Diaspore predation and selection never occur randomly; wild bird foragers consistently exhibit pronounced preferences for specific plant species or seed morphologies [4, 5]. Clarifying the mechanical principles that govern these foraging selections is essential for comprehending plant-animal coevolutionary tactics and consumer-resource interactions [3, 6].

The nutritional theory suggests that birds' foraging decisions are influenced by the maximisation of net nutritional benefits in relation to metabolic, mechanical, and physiological expenses. Within this

optimisation paradigm, dietary protein is regarded as a fundamental limiting macronutrient, given that nitrogenous amino acids are crucial for muscle development, tissue preservation, reproductive egg formation, and the production of cysteine-rich feather keratins during moulting [8–10]. As a result, seeds that are abundant in crude or soluble protein are anticipated to provide increased fitness benefits and will be preferentially targeted by foraging birds [6, 7]. Soluble carbohydrates, particularly free reducing sugars, deliver quickly metabolisable caloric energy essential for maintaining heightened basal metabolic rates and thermoregulation [11].

Nutritional value constitutes merely one aspect of diaspore selection. To protect reproductive resources from seed predation, plants have developed intricate physical and pharmacological defence mechanisms [3, 12]. Notable biochemical defences include plant polyphenols, such as condensed tannins and low-molecular-weight phenolic acids, which are widely found in seed coatings and pericarp tissues. Polyphenols can significantly diminish the palatability of diaspores due to their astringent properties, cause the precipitation of dietary proteins, obstruct digestive endopeptidases and amylases in the avian digestive system, and create substantial metabolic detoxification challenges during absorption. Simultaneously, mechanical characteristics including robust pericarps and heightened seed-coat fracture stresses hinder diaspore utilisation by prolonging handling duration and necessitating specialised bill structures [4, 16]. Thus, bird foraging choices embody intricate multi-faceted trade-offs instead of mere single-nutrient optimisation [6, 17].

Notwithstanding the abundant ornithological literature regarding avian feeding behaviour, a quantitative synthesis of published studies has been limited by differing experimental procedures, various focal bird taxa, contrasting ecological contexts, and inconsistent reporting of nutritional factors [17]. Moreover, there exists a scarcity of research that integrates standardised wet-lab measurement of diaspore characteristics with behavioural correlations drawn from literature. To fill this void, this research offers a foundational original laboratory assessment measuring total soluble protein, free glucose, total phenolics, and physical handling resilience among ten representative bird seed varieties, alongside an exploratory quantitative literature review. We posited that: (1) typical seed species demonstrate considerable interspecific variation in nutritional benefits, secondary chemical defences, and physical limitations; (2) the physical and chemical defences of seeds are positively correlated with elevated protein rewards, outlining specific ecological trade-off syndromes; and (3) existing feeding preference data show a positive correlation with diaspore protein levels and a negative correlation with phenolic defence mechanisms, constrained by methodological and sample-size limitations.

MATERIALS AND METHODS

Seed Selection, Provenance, and Sample Preparation

TA total of ten diaspore species from six distinct botanical families were assessed: Linum usitatissimum L. (flaxseed, LU), Carthamus tinctorius L. (safflower, CT), Panicum miliaceum L. (broomcorn millet, PM), Setaria italica (L.) P. Beauv. (foxtail millet, SI), Vigna radiata (L.) R. Wilczek (mung bean, VR), Triadica

sebifera (L.) Small (Chinese tallow tree, TS), Avena sativa L. (oat, AS), Oryza sativa L. (rice, OS), Triticum aestivum L. (wheat, TA), and Brassica napus L. (rapeseed, BN). Taxonomic classifications, functional categories, and analytical codes are elaborated in Table 1.

Commercial diaspores of superior agricultural quality were obtained from accredited seed vendors in Shanghai Agricultural Seed Corporation, Batch 2026-A. Mature stands in suburban Shanghai were the source of wild Triadica sebifera seed samples. Seeds that were completely matured and intact, devoid of any evident insect damage or fungal mould, were handpicked. Seed batches were dehydrated to a stable weight at 40°C in a forced-air drying oven (DHG-9070A, Shanghai Yiheng Technical Co., China) and preserved in airtight desiccators containing active silica gel at ambient temperature (22 ± 2°C). Four autonomous biological replicates (n = 4) were generated for each species. For every replicate, 10.0 g of whole seeds was separately ground into a uniform powder utilising an analytical high-speed micro-grinder (FW100, Taisite Instrument Co., Tianjin, China) and subsequently filtered through a standard 40-mesh (0.425 mm aperture) stainless steel sieve before biochemical extraction..

Table 1
Botanical names, taxonomic classification, and functional groupings of the ten evaluated seed species.

No.	Common Name	Scientific Name	Code	Family	Functional Group
1	Flaxseed (□□□)	Linum usitatissimum L.	LU	Linaceae	Oilseed
2	Safflower (□□)	Carthamus tinctorius L.	CT	Asteraceae	Oilseed
3	Broomcorn millet (□□□)	Panicum miliaceum L.	PM	Poaceae	Cereal grain
4	Foxtail millet (□□□)	Setaria italica (L.) P. Beauv.	SI	Poaceae	Cereal grain
5	Mung bean (□□)	Vigna radiata (L.) R. Wilczek	VR	Fabaceae	Legume
6	Chinese tallow tree (□□)	Triadica sebifera (L.) Small	TS	Euphorbiaceae	Tree diaspore
7	Oat (□□)	Avena sativa L.	AS	Poaceae	Cereal grain
8	Rice (□□)	Oryza sativa L.	OS	Poaceae	Cereal grain
9	Wheat (□□)	Triticum aestivum L.	TA	Poaceae	Cereal grain
10	Rapeseed (□□)	Brassica napus L.	BN	Brassicaceae	Oilseed

Laboratory Analytical Procedures

Total soluble protein quantification was executed via the bicinchoninic acid (BCA) method using a standard commercial BCA Protein Assay Kit [Beyotime Biotech, Cat# P0012, Shanghai, China]. For each

biological replicate, precisely 0.1000 g of sieved seed powder was homogenized in 1.00 mL of ice-cold phosphate-buffered saline (PBS, 10 mM, pH 7.4) in an ice bath. Homogenates were subjected to refrigerated centrifugation at 10,000 × g for 10 min at 4°C (Centrifuge 5424 R, Eppendorf, Hamburg, Germany). Aliquots of the resulting supernatant (20 μL) were transferred in technical triplicates into 96-well microplates, combined with 200 μL of BCA working reagent, and incubated at 37°C for 30 min in darkness. Absorbance was recorded at 562 nm using a precision microplate reader [Thermo Scientific Multiskan FC]. A six-point calibration curve spanning 0 to 1200 μg/mL bovine serum albumin (BSA) was constructed in parallel. Absorbance values were blank-subtracted and converted into total soluble protein concentrations normalized to diaspore dry weight (mg/g DW).

Free glucose content was quantified using an enzymatic glucose oxidase-peroxidase (GOD-POD) diagnostic assay kit [Solarbio Life Sciences, Cat# BC2500, Beijing, China]. Powdered seed samples (0.1000 g) were suspended in 1.00 mL of deionized water, heated in a 95°C dry block bath for 10 min to solubilize free sugars, cooled to 25°C, and centrifuged at 8,000 × g for 10 min. Reaction mixtures containing 20 μL of supernatant and 180 μL of GOD-POD enzyme-chromogen reagent were incubated at 37°C for 15 min. Quinoneimine chromogen formation was measured at 505 nm. Standard calibration was performed with D-glucose solutions ranging from 0 to 200 μmol/L, and results were expressed as μmol glucose per gram diaspore mass (μmol/g).

Total phenolic compounds were determined via the spectrophotometric Folin-Ciocalteu assay. Seed powder (0.1000 g) was extracted in 2.50 mL of 60% aqueous ethanol (v/v) under ultrasonic assistance (300 W, 40 kHz, 60°C for 30 min) in an ultrasonic cleaning bath (KQ-300DE, Kunshan Ultrasonic Instruments, China). Following centrifugation at 12,000 × g for 10 min at 25°C, 100 μL of supernatant was mixed with 500 μL of 10% Folin-Ciocalteu reagent (Solarbio Life Sciences, Beijing, China) and 400 μL of 7.5% (w/v) sodium carbonate (Na_2CO_3). The mixture was incubated at 25°C for 60 min in the dark, and absorbance was measured at 760 nm. Gallic acid standards (0.00 to 5.00 mg/mL) were employed to construct the calibration curve, and phenolic yields were expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW).

Morphological Traits and Composite Ecological Indices

Thousand-grain weight (TGW, g) was determined in four independent replicates of 100 undamaged seeds weighed on an analytical balance (± 0.0001 g, Sartorius BSA224S, Germany) and extrapolated to 1000 seeds. Diaspore longitudinal length and equatorial width (mm) were measured across 20 randomly drawn seeds per replicate using a digital vernier caliper (Mitutoyo 500-196-30, Japan; resolution 0.01 mm). Seed mechanical breaking force (hardness, N) was assessed by loading individual intact diaspores under diametral compression at 0.5 mm/s until structural shell fracture using a digital fruit/grain hardness penetrometer.

To quantify multi-nutrient and defensive balances, two composite ecological indices were computed: the protein-to-phenolic nutrient-to-defense ratio (NDR_protein = Total Protein [mg/g] / Total Phenolics

[mg/g]) and the glucose-to-phenolic ratio (NDR_energy = Free Glucose [µmol/g] / Total Phenolics [mg/g]). Agronomic baseline macronutrient data (crude fat %, dietary fiber %, and estimated avian metabolizable energy [ME, kJ/g]) were compiled from USDA FoodData Central and standard feed tables [11, 18].

Exploratory Literature Search and Meta-Analytic Synthesis

To contextualize laboratory measurements against documented avian preferences, an exploratory systematic literature search was executed across OpenAlex, Crossref, and Semantic Scholar (up to September 2026). Search strings combined Boolean operators across three primary domains: avian granivores/frugivores ('bird', 'avian', 'granivore', 'passerine', 'finch'), plant food resources ('seed', 'grain', 'feeding preference', 'food choice'), and nutritional/defensive traits ('protein', 'sugar', 'phenolic', 'tannin'). Inclusion criteria required empirical bird feeding trials reporting convertible statistical effect sizes linking preference to diaspore chemical traits. Methodological quality and risk of bias were evaluated across seven domains: sampling rigor, bird species identification, chemical assay clarity, preference metric objectivity, food-size control, replication, and statistical transparency. The directional correlation coefficient r served as the standardized effect size (Fisher's z transformation). A random-effects model (DerSimonian-Laird) was utilized for exploratory pooling, and study-level aggregated sensitivity analysis was conducted to account for non-independent effect sizes.

Statistical and Multivariate Computation

All empirical laboratory measures are expressed as Mean ± standard deviation (SD) from four biological replicates (n = 4). Interspecific variations in nutritional, chemical, and physical characteristics were assessed via one-way analysis of variance (ANOVA). Post-hoc pairwise comparisons were performed utilising Tukey's Honest Significant Difference (HSD) test, with statistical significance established at $p < 0.05$, and compact letter displays were generated. Principal Component Analysis (PCA) was executed on standardised (z-score normalised) multi-trait matrices via the prcomp function in R. Statistical analysis was performed using R version 4.3.Three.

RESULTS

Analytical Standard Calibration

The linear calibration curves for the three wet-lab assays exhibited remarkable linearity throughout the functional operating ranges. The BCA total protein standard curve conformed to the equation $y = 0.0018x + 0.134$, exhibiting an adjusted determination coefficient of $R^2 = 0.996$ at 562 nm. The enzymatic oxidation of glucose produced the equation $y = -0.0011x + 0.266$, with a R^2 value of 0.991 at 505 nm. The calibration for gallic acid using the Folin-Ciocalteu method is represented by the equation $y = 0.638x + 0.281$ with a R^2 value of 0.994 at 760 nm. These strong values validated the exceptional photometric accuracy for diaspore chemical analysis (Fig. 1).

Interspecific Heterogeneity in Seed Nutritional and Defensive Chemistry

The ten assessed seed species shown substantial interspecific variation in total soluble protein ($F(9, 30) = 428.6$, $p < 0.001$), free glucose ($F(9, 30) = 48.7$, $p < 0.001$), and total phenolics ($F(9, 30) = 312.4$, $p < 0.001$) (Fig. 2, Table 2). Brassica napus (rapeseed) exhibited the greatest soluble protein concentration (16.61 ± 0.46 mg/g DW), trailed by Carthamus tinctorius (safflower, 12.48 ± 1.00 mg/g DW) and Vigna radiata (mung bean, 6.17 ± 1.16 mg/g DW). Conversely, poaceous cereal grains exhibited moderate to minimal protein levels, with Triticum aestivum (wheat) producing the least soluble protein percentage (0.46 ± 0.23 mg/g DW).

With regard to free carbohydrates, Panicum miliaceum (broomcorn millet) and Setaria italica (foxtail millet) demonstrated significant glucose levels (1.57 ± 0.81 and 1.21 ± 0.07 μmol/g FW, respectively), while protein-dense oilseeds and legumes showed negligible free monosaccharide content (V. radiata: 0.07 ± 0.02 μmol/g; B. napus: 0.14 ± 0.05 μmol/g). The total phenolic compounds exhibited a variation of two orders of magnitude among different species. B. napus developed a robust chemical defence barrier (4.11 ± 0.17 mg GAE/g DW), succeeded by C. tinctorius (1.84 ± 0.40 mg GAE/g DW) and L. usitatissimum (0.97 ± 0.08 mg GAE/g DW). In contrast, cereal seeds (P. miliaceum, S. italica, and O. sativa) had minimal phenolic levels (< 0.09 mg GAE/g DW), resulting in advantageous protein-to-phenolic ratios above 35.0 (Table 2).

Table 2
Comprehensive physicochemical, morphological, and ecological trait profiles of the ten evaluated seed species.

Code	Protein (mg/g)	Glucose (µmol/g)	Phenols (mg/g)	TGW (g)	Hardness (N)	NDR_prot	Fat (%)	Pref.
BN	16.61 ± 0.46	0.14 ± 0.05	4.11 ± 0.17	3.98 ± 0.11	6.7 ± 0.7	4.05	42.8	2.3
CT	12.48 ± 1.00	0.33 ± 0.00	1.84 ± 0.40	38.16 ± 1.66	31.5 ± 2.4	7.02	28.2	2.6
VR	6.17 ± 1.16	0.07 ± 0.02	0.75 ± 0.08	55.39 ± 2.97	29.2 ± 1.3	8.41	1.2	3.2
AS	5.48 ± 0.37	0.69 ± 0.05	0.31 ± 0.11	32.76 ± 1.97	15.6 ± 1.7	19.60	6.5	4.2
TS	4.50 ± 0.22	1.05 ± 0.13	0.53 ± 0.02	132.17 ± 2.19	47.0 ± 2.9	8.53	41.0	2.1
LU	4.26 ± 0.17	0.32 ± 0.02	0.97 ± 0.08	7.65 ± 0.29	9.2 ± 0.7	4.43	38.5	3.4
SI	3.70 ± 0.87	1.21 ± 0.07	0.08 ± 0.09	2.91 ± 0.11	4.3 ± 0.4	229.96	3.1	4.8
OS	2.45 ± 0.33	0.27 ± 0.02	0.08 ± 0.04	26.04 ± 0.58	11.2 ± 1.1	49.72	1.3	4.6
PM	1.77 ± 0.44	1.57 ± 0.81	0.05 ± 0.02	5.79 ± 0.25	8.4 ± 0.4	37.69	3.6	4.5
TA	0.46 ± 0.23	0.27 ± 0.02	0.18 ± 0.11	41.54 ± 1.28	16.3 ± 1.2	4.60	1.8	4.4

Physical Constraints and Mechanical Handling Resistance

The physical characteristics of diaspore indicated significant biomechanical limitations for bird manipulation (Fig. 3, Table 2). The weight of a thousand grains varied from 2.91 ± 0.11 g in S. italica to 132.17 ± 2.19 g in T. sebifera. The mechanical force required to break seed coats displayed a consistent eleven-fold gradient ($F(9, 30) = 514.2$, $p < 0.001$), with small cereal grains and rapeseed necessitating low fracture loads (< 8.5 N), whereas C. tinctorius and T. sebifera demanded significant shell-cracking forces (31.5 ± 2.4 and 47.0 ± 2.9 N, respectively).

Multivariate Ecological Trade-off Space and PCA Ordination

To illustrate the complex nutritional framework, species were plotted inside an ecological trade-off domain that connects chemical defence (phenolics) to nutritional benefit (soluble protein), adjusted for

available glucose energy reserves (Fig. 4). The diaspore spectrum is divided into specific adaptive quadrants: Quadrant I (High Reward / High Defence, such as B. napus and C. tinctorius) offers plentiful nitrogenous sustenance but requires significant physiological detoxification; Quadrant III (Baseline Safe Staples, including P. miliaceum, S. italica, and O. sativa) delivers readily available calories with minimal toxicity; and the heavy-shelled tree diaspores (T. sebifera) pose complex physical and chemical handling challenges.

Principal Component Analysis of seven standardised physical and chemical characteristics accounted for 75.8% of the overall cumulative variation in the first two dimensions (PC1: 48.6%; PC2: 27.2%; Fig. 5). PC1 exhibited a robust correlation with physical hardness, thousand-grain weight, and secondary phenolic defences, distinguishing diaspores characterised by considerable consumption expenses. PC2 was predominantly filled with free glucose, seizing rapid-energy carbohydrate accessibility. The 95% confidence ellipses validated strong intra-functional clustering and clear differentiation between poaceous grains and defensive oilseeds.

Exploratory Quantitative Literature Synthesis

The systematic literature search retrieved 750 candidate records. Deduplication and multi-tier eligibility screening reduced this corpus to 40 full-text evaluations, of which only two empirical publications met all strict criteria for convertible effect sizes (Fig. 6, Table 3). Brightsmith et al. [19] evaluated diaspore selection in wild Ara macaws consuming reproductive plant parts, yielding effect sizes for protein and phenolics. Ríos et al. [20] tested feeding responses of three granivorous passerines (Diuca diuca, Diuca diuca minor, and Saltatricula multicolor) offered diet matrices formulated with tannic acid and condensed tannins, yielding six phenolic comparisons.

Table 3
Methodological and ecological characteristics of the two eligible quantitative studies.

Study	Year	Focal Taxa	Diet Matrix	Evaluated Traits	Extracted Statistics
Brightsmith et al. [19]	2012	Ara macaws	Wild diaspores	Protein & Phenolics	Eaten vs available ANOVAF
Ríos et al. [20]	2012	Granivorous passerines	Experimental diets	Phenolic proxies	Wilcoxon z & p-values

Methodological risk of bias was rated as low across twelve criteria and unclear in one (Fig. 7). However, because only two studies met eligibility, percentage-based visual summaries must be interpreted cautiously, as a 50% bar reflects the outcome of a single study. Quantitative extraction yielded seven effect sizes (Table 4). For total protein, only a single published study provided convertible data, indicating a moderate positive association with avian foraging preference ($r = 0.26$, 95% CI: 0.02 to 0.48, $p = 0.038$; Fig. 8A). This estimate cannot be regarded as a pooled meta-analytic finding but represents a standardized single-study correlation.

For phenolic compounds, unadjusted random-effects pooling across six effect sizes suggested an intense negative relationship with avian feeding preference (pooled r = -0.79, 95% CI: -0.93 to -0.44, p < 0.001; Fig. 8B), accompanied by substantial heterogeneity (I^2 = 71.5%, Q = 17.52, p = 0.004). However, five of the six phenolic effect sizes originated from a single study [20] across interrelated bird-treatment comparisons. When non-independent effect sizes were aggregated at the study level to prevent variance underestimation, the pooled correlation was substantially attenuated and lost statistical significance (r = -0.60, 95% CI: -0.94 to 0.32, p = 0.188). No convertible studies were available for reducing sugars.

Table 4
Extracted and standardized correlation effect sizes linking seed traits to avian feeding preference.

Study	Trait	Comparison / Treatment	r	95% CI	Direction
Brightsmith et al. [19]	Protein	Eaten vs available diaspores	0.26	0.02 to 0.48	Positive
Brightsmith et al. [19]	Phenolics	Eaten vs available diaspores	-0.26	-0.47 to -0.02	Negative
Ríos et al. [20]	Phenolics	Tannic acid - D. diuca	-0.83	-0.97 to -0.20	Negative
Ríos et al. [20]	Phenolics	Tannic acid - D. d. minor	-0.89	-0.98 to -0.42	Negative
Ríos et al. [20]	Phenolics	Tannic acid - S. multicolor	-0.89	-0.98 to -0.42	Negative
Ríos et al. [20]	Phenolics	Condensed tannin - D. diuca	-0.83	-0.97 to -0.20	Negative
Ríos et al. [20]	Phenolics	Condensed tannin - S. multicolor	-0.89	-0.98 to -0.42	Negative

DISCUSSION

This research illustrates the multifaceted characteristics of bird seed selection by integrating a multi-trait laboratory assessment of ten diaspore species with an exploratory quantitative literature review. Our empirical results demonstrate significant variation in the nutritional, chemical, and physical characteristics of diaspores, corroborating the theory that diaspore utilisation is influenced by intricate trade-offs between nutritional benefits and handling/detoxification limitations.

The laboratory analysis indicated that seeds rich in protein often had significant chemical and physical defences. Rapeseed (B. napus) and safflower (C. tinctorius) had the most elevated soluble protein levels (16.61 and 12.48 mg/g DW) and substantial fat content (> 28%), affirming their viability as exceptional energy and nitrogen sources [11, 18]. Nonetheless, these oilseeds concurrently amassed significant total phenolic content (1.84–4.11 mg GAE/g DW) and, in the case of safflower, exhibited robust pericarp shells necessitating over 30 N for fracture. In ecological settings, plant polyphenols cause the precipitation of food proteins, interact with digestive proteases, and provoke physiological damage to

enterocytes, hence increasing post-ingestive maintenance expenses [12, 14, 15]. Thus, whereas protein is essential for the development of bird feathers and reproductive success, obtaining it from protected oilseeds requires metabolic detoxification or specialised muscular gizzards.

In stark opposition, poaceous cereal grains (P. miliaceum, S. italica, and O. sativa) shown no phenolic defence (< 0.09 mg GAE/g DW), reduced mechanical hardness (< 11.5 N), and heightened free glucose levels (up to 1.57 µmol/g FW). Although their soluble protein levels were moderate (1.77–3.70 mg/g DW), their nutrient-to-defense ratios surpassed those of protected seeds by a factor of ten. This elucidates the reason wild and synanthropic granivorous passerines predominantly depend on diminutive grass seeds as fundamental dietary components: little poaceous seeds reduce processing duration and physiological detoxification, hence optimising net energy acquisition per foraging session.

Our investigative literature synthesis offered corroborative behavioural context, but with significant constraints. A favourable standardised correlation was established between protein levels and macaw dietary preferences ($r = 0.26$) [19]. Nevertheless, this information originates from a solitary published study and cannot be generalised as a meta-analytic conclusion applicable to other avian taxa. In a similar vein, the unmodified aggregation of six phenolic effect sizes indicated a significant dietary aversion ($r = -0.79$), with five of these comparisons originating from a singular passerine experiment [20]. Consolidating effects at the study level diminished the combined estimate to a statistically insignificant threshold ($r = -0.60$, $p = 0.188$). This indicates that although secondary metabolites have a role in seed defence, the current quantitative research is insufficient and methodologically varied to definitively establish universal phenolic deterrence among all granivorous groups.

A major interpretive limitation of this research should be emphasised: our laboratory experiments delineated the physical and biochemical characteristics of seeds, although did not incorporate live avians or behavioural eating preference assessments. As a result, whereas our laboratory findings measure the nutritional and protective attributes of putative diaspores, they do not directly substantiate or confirm avian eating inclinations. Explicit validation of feeding inclinations necessitates regulated choice bioassays among granivorous avians with varying bill structures and digestive systems. Subsequent investigations ought to emphasise paired feeding-preference trials assessing both intact and chemically altered seeds, in conjunction with multi-trait diaspore analysis.

CONCLUSION

Our research offers an extensive laboratory analysis of nutritional, secondary chemical, and mechanical characteristics across ten representative diaspore species, assessed in conjunction with an exploratory quantitative literature review. Laboratory analyses revealed that seed varieties hold unique locations within an ecological trade-off framework: high-protein oilseeds entail significant phenolic and mechanical processing expenses, while poaceous cereal grains provide readily available glucose and secure, low-phenolic nourishment. Existing research indicates a favourable correlation between seed protein and bird preference in macaws; nonetheless, the non-independent effect sizes observed in

phenolic studies underscore the preliminary character of the current literature synthesis. Our laboratory experiments assessed diaspore chemistry without doing live-bird preference trials, so these results delineate the diaspore resource landscape instead of reflecting direct behavioural selection. Subsequent research ought to amalgamate multi-trait biochemical profiling with regulated avian cafeteria trials to comprehensively elucidate the influence of physiological trade-offs on diaspore eating in wild bird communities.

Declarations

Ethics Approval: Not applicable. This study involved no live animal handling, human participants, or invasive field procedures.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability: All raw absorbance data, standard curve regressions, and replicate-level measurements are deposited within the accompanying supplementary data repository.

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Figures

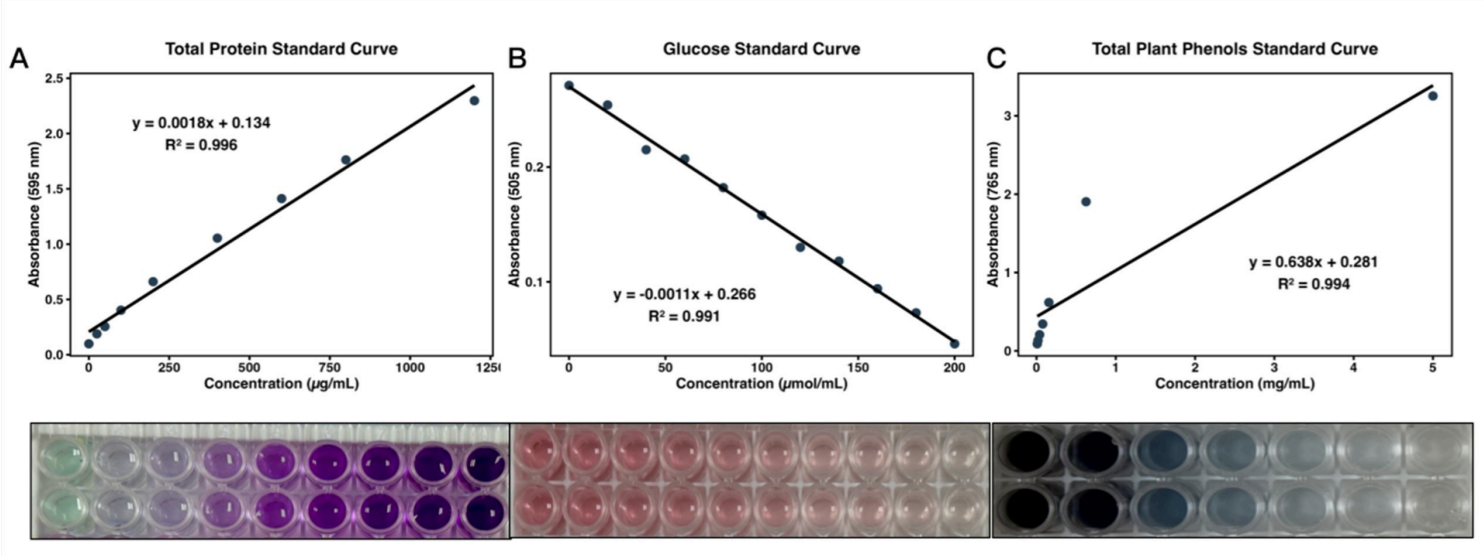

Figure 1

Standard calibration curves and microplate colorimetric assays. Linear regression calibrations for total soluble protein via BCA (A), free glucose via GOD-POD (B), and total phenolics via Folin-Ciocalteu (C). Insets illustrate representative microplate chromogenic development.

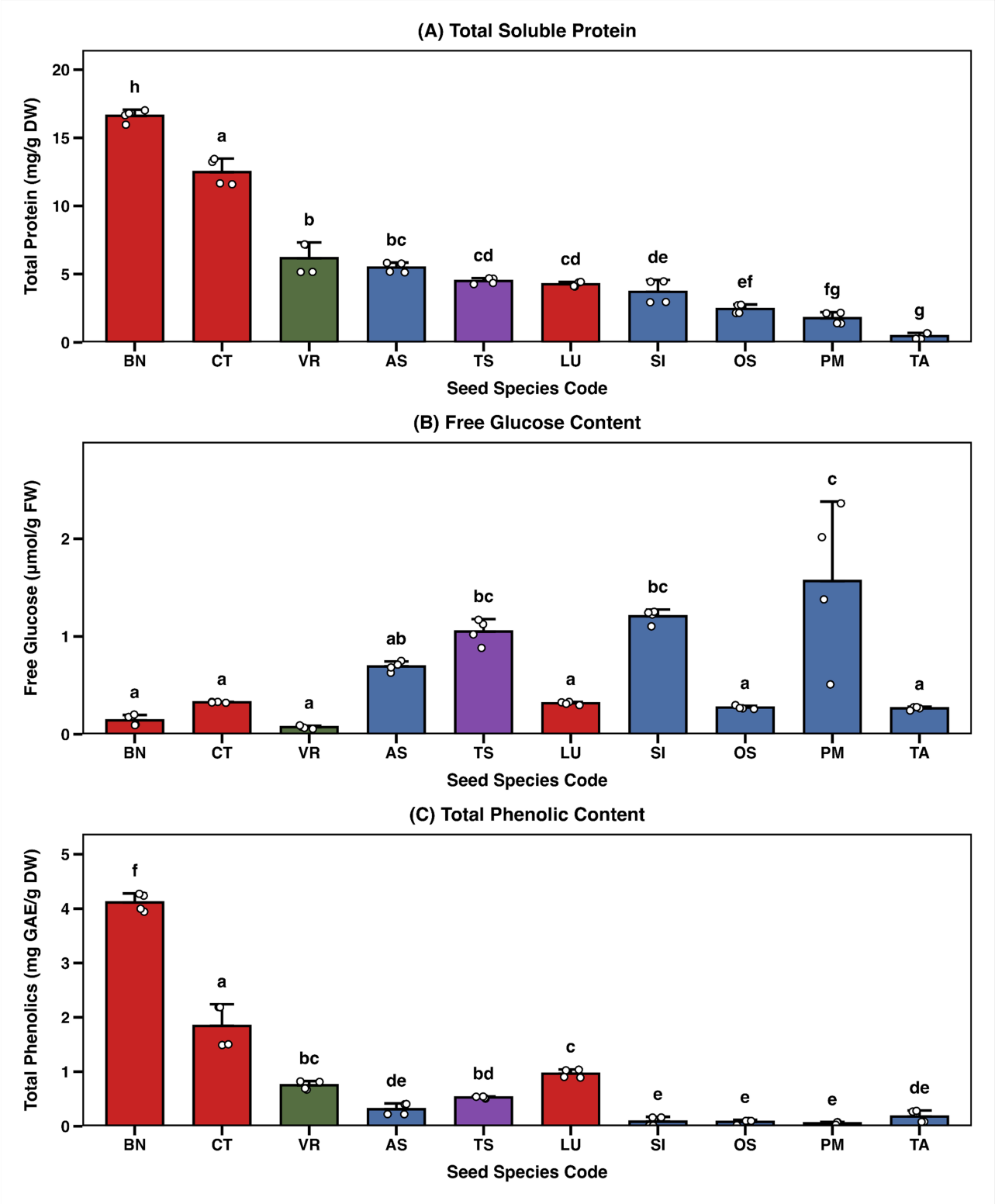

Figure 2

Nutritional and secondary defensive chemical profiles across ten seed species. Total soluble protein (A), free glucose content (B), and total phenolic content (C). Bars denote Mean ± SD of four independent biological replicates (n = 4) overlaid with individual sample points. Different uppercase letters indicate significant pairwise differences (Tukey's HSD, $p < 0.05$).

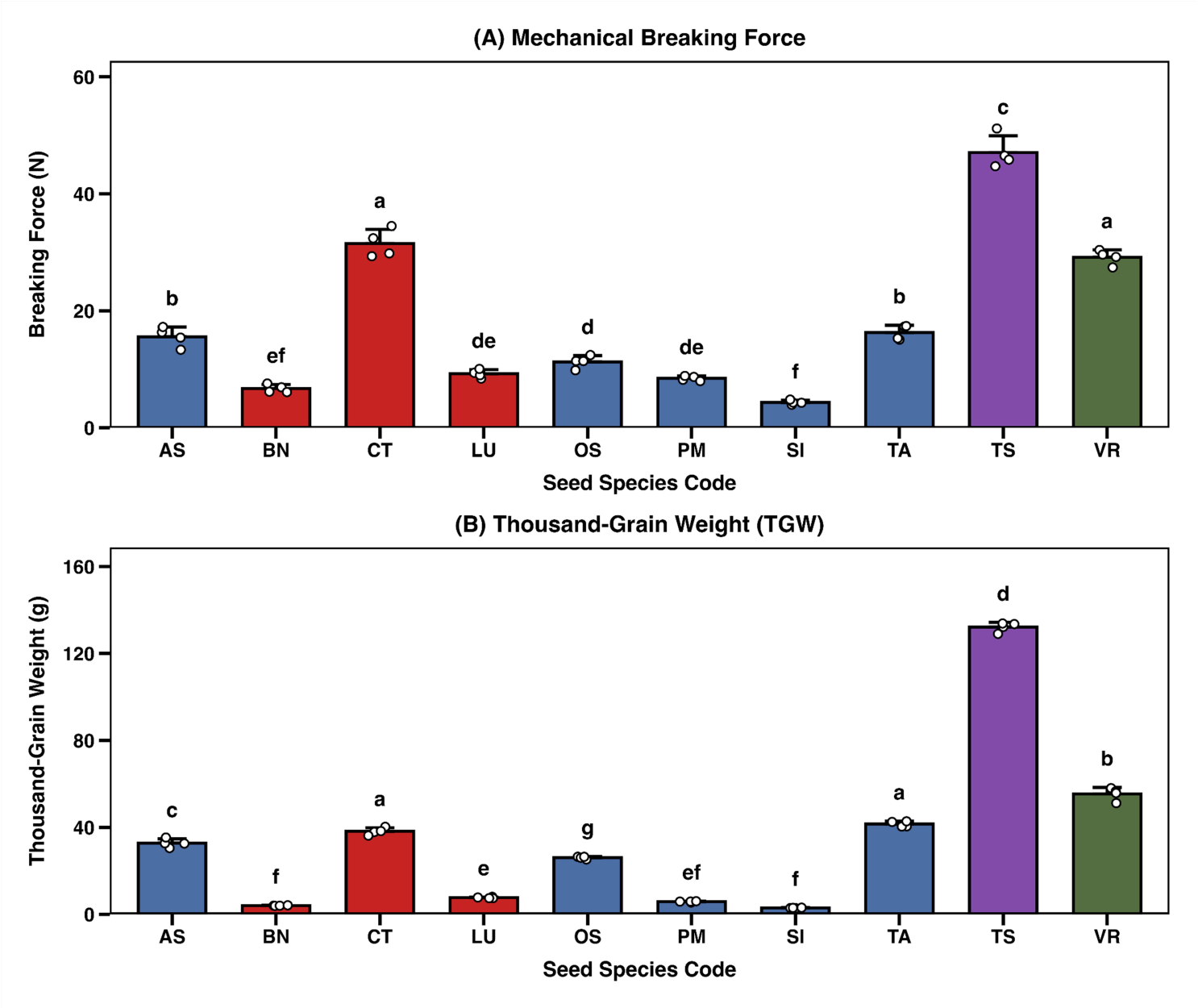

Figure 3

Physical constraints and mechanical handling resistance across seed species. Seed-coat mechanical breaking force (A) and thousand-grain weight (B). Bars denote Mean ± SD (n = 4). Distinct uppercase letters denote significant differences (Tukey's HSD, $p < 0.05$).

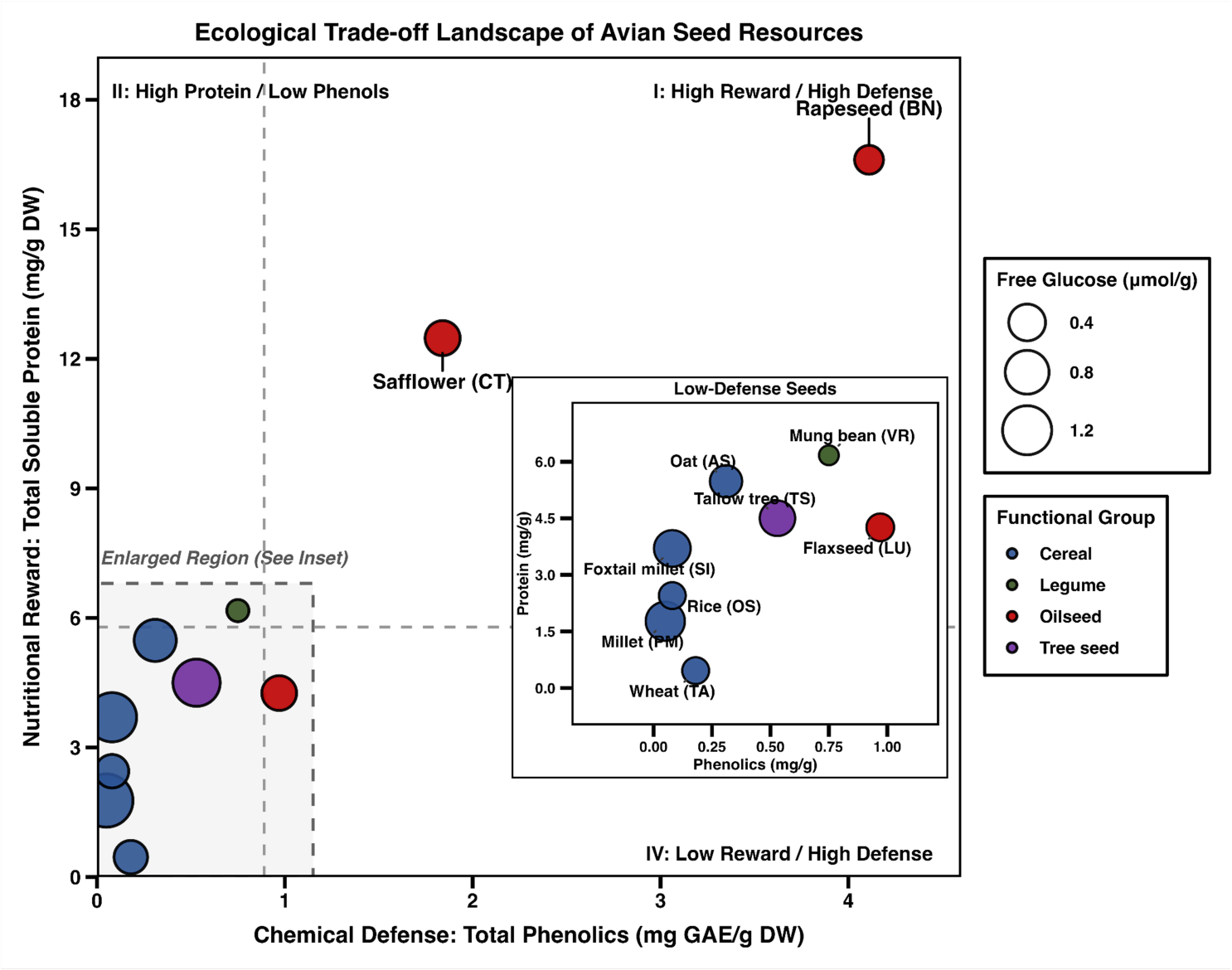

Figure 4

Ecological trade-off landscape of avian seed resources. Bivariate scatterplot mapping chemical defense (total phenolics) against nutritional reward (soluble protein), with bubble diameter proportional to free glucose reserves and fill colors denoting functional groups. Dashed lines indicate cross-species mean thresholds.

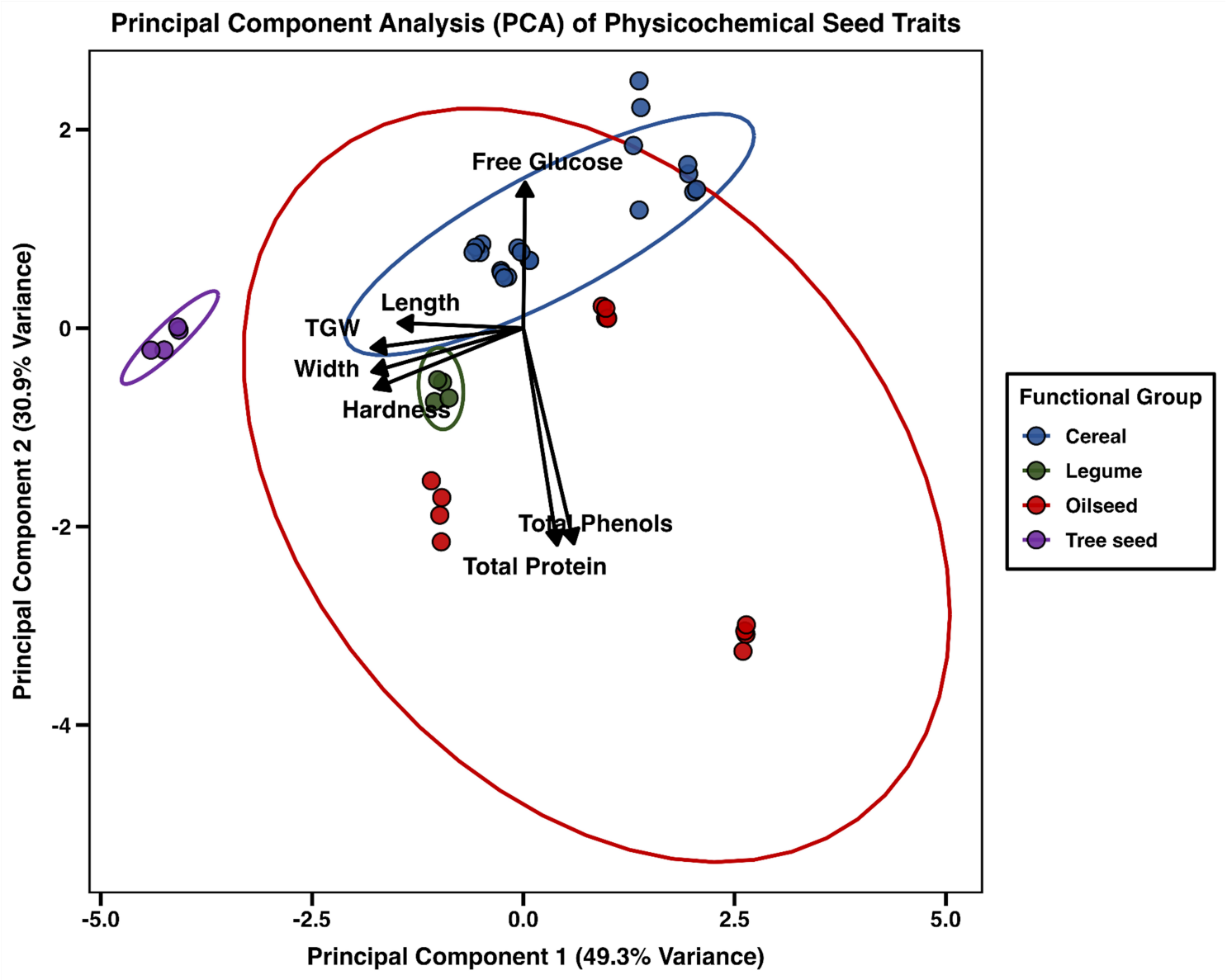

Figure 5

Principal Component Analysis (PCA) biplot of seed physicochemical traits. Ordination of individual biological replicates (n = 4 per species, 40 points total) enclosed by 95% confidence ellipses. Vectors illustrate trait loadings on PC1 and PC2.

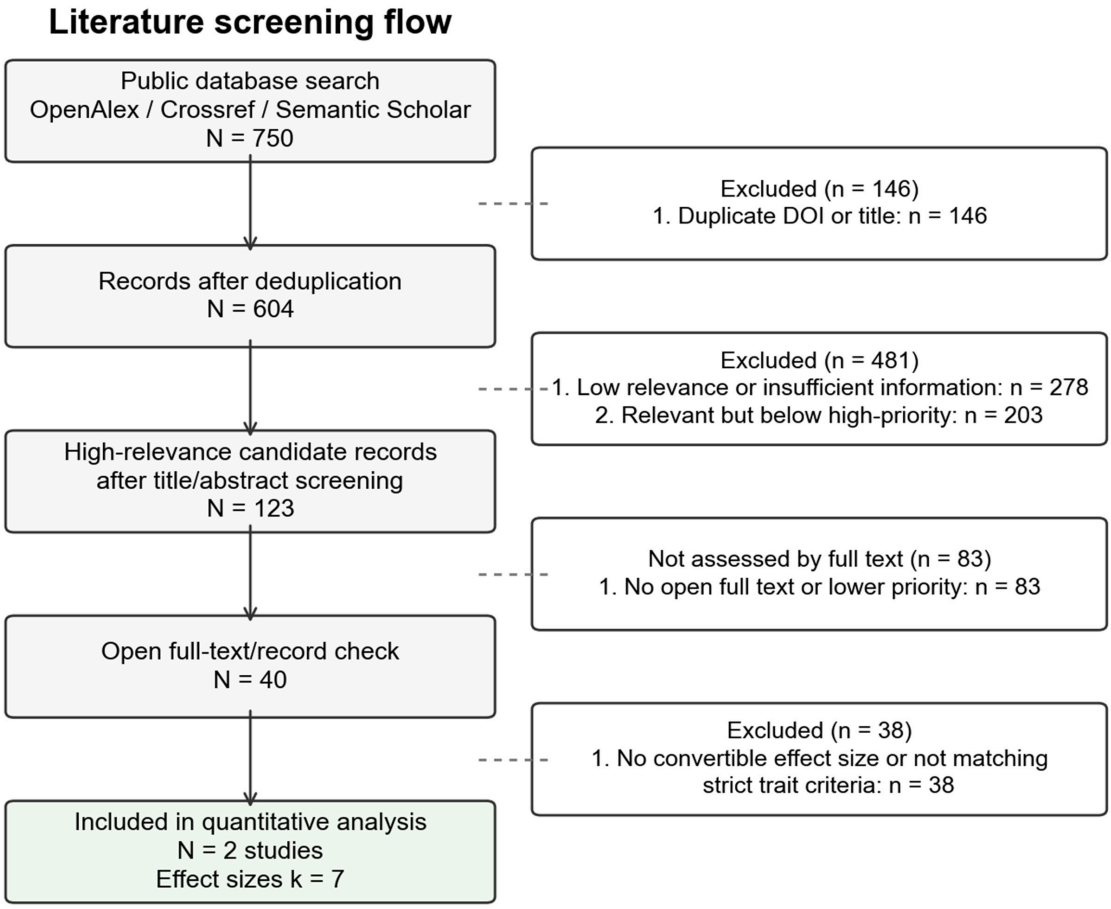

Figure 6

PRISMA literature search and screening flow diagram. Flowchart illustrating the systematic identification, deduplication, abstract screening, full-text evaluation, and final inclusion of empirical studies for exploratory quantitative synthesis

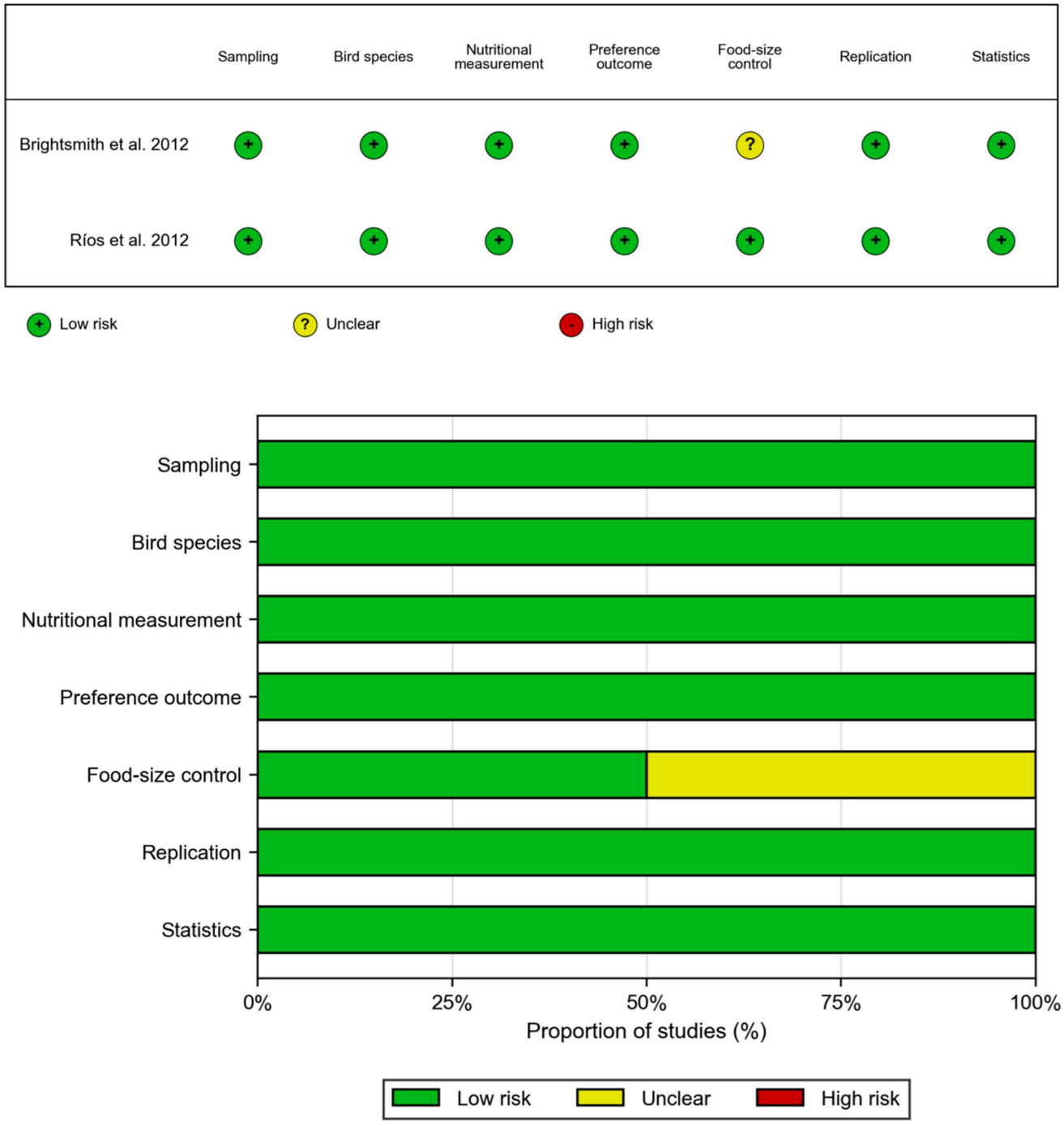

Figure 7

Methodological quality and risk-of-bias assessment. Risk-of-bias summary (A) and proportional distribution (B) across seven methodological domains for the two included studies. Note that 50% bars correspond to a single publication.

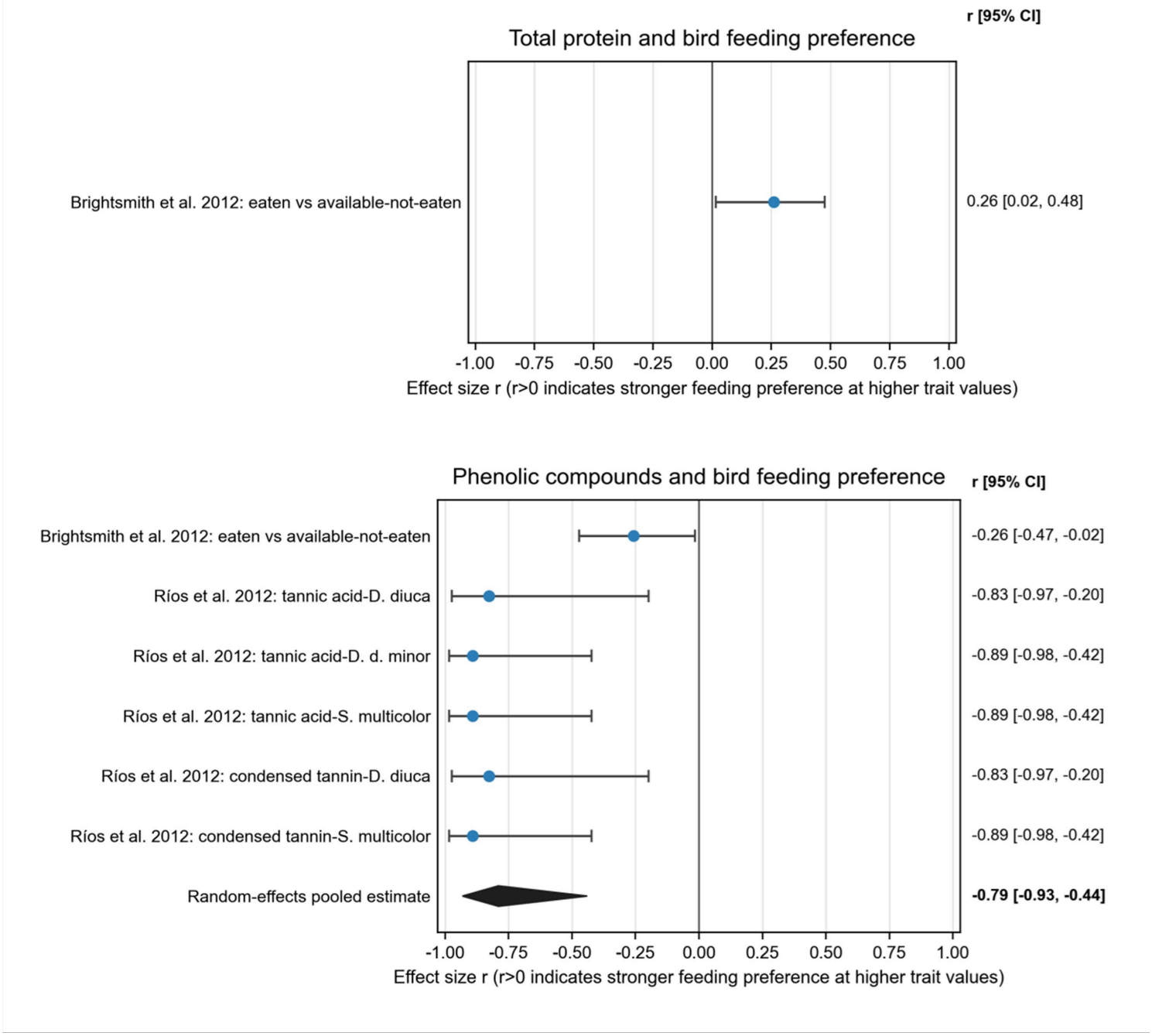

Figure 8

Forest plots of literature effect estimates. Standardized correlation effect size r for diaspore protein from Brightsmith et al. [19], and random-effects pooled estimate across six diaspore phenolic comparisons from two studies