

1 *Original Article*

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3 **Inconsistent relationships detected between seed size, shape, and persistence for**
4 **different plant functional groups in the Pannonian flora**

5 Running title: Seed size, seed shape, and seed persistence in the Pannonian flora

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7 Viktória Törő-Szjgyártó¹, Péter Török^{1,2,3}, Katalin Tóth¹, Hajnalka Málik-Roffa², Luis Roberto
8 Guallichico Suntaxi¹, Szilvia Madar², Gergely Kovacsics-Vári¹, Andrea McIntosh-Buday^{1,2},
9 Patricia Díaz Cando¹, Judit Sonkoly^{1,2*}

10

11 ¹ Department of Ecology, University of Debrecen, 1 Egyetem sqr., 4032 Debrecen, Hungary

12 ² HUN-REN-UD Functional and Restoration Ecology Research Group, 1 Egyetem sqr., 4032
13 Debrecen, Hungary

14 ³ Polish Academy of Sciences, Botanical Garden-Centre for Biological Diversity
15 Conservation in Powsin, Warszawa, Poland

16

17 Corresponding author's email address:

18 *judit.sonkoly@gmail.com

19

20 **ABSTRACT**

21 **Background and Aims**

22 Knowledge on seed persistence is vital from both theoretical and practical considerations
23 but directly collecting persistence data for many species is unfeasible. Therefore, there is a
24 need to identify traits associated with seed persistence, but studies about the effects of seed
25 size and shape on persistence yielded results varying across regions. We studied 392
26 species of the Pannonian flora (Central Europe) to assess (i) how seed mass and shape are
27 related to seed persistence, (ii) whether this relationship is consistent across plant

1 functional groups, and (iii) whether seed mass and shape are correlated in different
2 functional groups?

3 **Methods**

4 We collected data on the seed mass and persistence of species and performed
5 measurements to calculate their Seed Shape Index, with higher values indicating greater
6 deviation from sphericity. To account for phylogenetic non-independence, we analysed how
7 seed mass and Seed Shape Index affect persistence in all herbaceous species and
8 separately in four plant functional groups using phylogenetic logistic regressions. To test how
9 seed mass and shape are related to each other in these groups, we applied PGLS regression.

10 **Key Results**

11 Across all species, both seed mass and Seed Shape Index were negatively related to
12 persistence, with seed mass having a stronger association. The same relationship was
13 observed in forbs and short-lived species, but only seed shape was related to persistence in
14 graminoid species. The relationship between seed mass and seed shape also varied
15 between functional groups.

16 **Conclusions**

17 Consistent with many studies in other floras, both seed mass and shape were negatively
18 related to persistence in the Pannonian flora after accounting for phylogeny. However, only
19 seed shape was associated with persistence in graminoid species, suggesting that different
20 factors may be at play in forbs and graminoids. Therefore, future studies of this relationship
21 may need to treat and analyse graminoids separately.

22 **Key words:** Pannonian flora, persistence, plant functional groups, seed longevity, seed
23 mass, seed morphology, seed shape, seed size, seed weight, soil seed bank

24 **INTRODUCTION**

25 Studying soil seed banks is vital for understanding the dynamics of plant populations and
26 communities (Hopfensperger, 2007; Plue *et al.*, 2017, 2020) and how different species deal
27 with environmental heterogeneity and uncertainty (Long *et al.*, 2015; Gioria *et al.*, 2020). As

1 soil seed banks disperse genetic diversity and mortality risks in time, they strongly promote
2 the maintenance of plant populations (Gioria *et al.*, 2020). Persistent soil seed bank
3 formation decreases the risk related to reproductive failure during periods of adverse
4 environmental conditions, thereby constituting a bet-hedging strategy (Venable and Brown,
5 1988). In plant communities exposed to environmental change, for example climate change
6 or habitat isolation, persistent soil seed banks can decrease extinction risk and contribute
7 to population persistence (Stöcklin and Fisher, 1999; Rees *et al.*, 2002, Estrada *et al.*, 2015).
8 Soil seed banks also act as reserves of genetic variability (Levin, 1990; Aparicio *et al.*, 2002).
9 As persistent soil seed banks can contain seeds produced over multiple years and years with
10 different environmental conditions benefit different genotypes of a species, the seed bank
11 can provide a great diversity of seed genotypes adapted to varying environmental conditions
12 (Cabin, 1996). Therefore, persistent soil seed banks play a vital role in the resilience of plant
13 communities (Kiss *et al.*, 2018) and the maintenance of biodiversity through space and time
14 (Royo and Ristau, 2013). In spite of this, our knowledge on soil seed banks is still
15 disproportionately limited compared to the aboveground vegetation.

16 Seed persistence is essential for the formation of a persistent soil seed bank. It refers to the
17 ability of seeds to remain viable for a long time (Fenner and Thompson, 2005), allowing them
18 to persist in the soil until environmental conditions are favourable, thereby promoting
19 survival under changing or unpredictable conditions (e.g., del Cacho and Lloret, 2012,
20 Estrada *et al.*, 2015). Seed persistence, which primarily varies on a continuous scale, is
21 generally classified into discrete categories for simplicity and practical application. The
22 most widespread seed bank classification system distinguishes three categories: (i)
23 transient seeds (viable for <1 year), (ii) short-term persistent seeds (viable for 1–5 years), and
24 (iii) long-term persistent seeds (viable for ≥ 5 years) (Thompson *et al.*, 1997). However, the
25 boundaries are not always clear, especially between the latter two categories (Thompson *et al.*,
26 1993). Therefore, distinguishing between transient and persistent species – without
27 differentiating between short-term and long-term persistence – is a reasonable approach for
28 general discussions and broad analyses (see e.g., Bekker *et al.*, 1998; Funes *et al.*, 1999;
29 Gioria *et al.*, 2020). This distinction is especially important because it determines whether

1 seeds of a species are able to accumulate over multiple seasons and therefore disperse in
2 time (Baskin and Baskin, 2014).

3 The ability to distinguish species with transient versus persistent seeds is not only valuable
4 for answering a wide range of fundamental questions in vegetation science and population
5 biology (Saatkamp *et al.*, 2009), but it is crucial for the success and feasibility of restoration
6 projects as well (von Blanckenhagen and Poschlod, 2005; Török *et al.*, 2018). Unfortunately,
7 obtaining direct data on seed persistence is challenging and time-consuming (Cerabolini *et*
8 *al.*, 2003; Jaganathan *et al.*, 2019). Soil seed bank analyses, whether using the seedling
9 emergence or seed extraction method, require considerable effort. Moreover, the results are
10 not necessarily conclusive, and the findings of different studies are frequently contradictory
11 (Cerabolini *et al.*, 2003). There are other methods to assess seed longevity more accurately,
12 but they are either costly and challenging (such as carbon-dating viable seeds, e.g., Moriuchi
13 *et al.*, 2000), or take a particularly long time (such as burial experiments, e.g., Telewski and
14 Zeevaart, 2002; Pakeman *et al.*, 2012). Therefore, collecting direct seed persistence data for
15 many species is rather unrealistic. Based on the above considerations, reliably predicting
16 the ability of seeds to persist in the soil is the only realistic option for obtaining information
17 on the transient versus persistent nature of a wide range of species. Since such information
18 is highly needed from both theoretical and practical conservation perspectives (e.g.,
19 Saatkamp *et al.*, 2009; Kalamees *et al.*, 2012), investigating which attributes correlate with
20 seed persistence and how these attributes can improve the reliability of predictions is vitally
21 important.

22 Seed mass is the most frequently measured trait of seeds (Carta *et al.*, 2024) and it is
23 considered to have an exceptionally large functional importance, as it is connected to
24 several processes and plant characteristics, such as dispersal distance, light detection,
25 seedling establishment, seed predation, or the number of seeds produced (summarised for
26 example by Moles, 2018 and Carta *et al.*, 2024). Seed shape can also influence many
27 processes such as soil penetration (Chambers *et al.*, 1991), fire tolerance (Ruprecht *et al.*,
28 2015), or the chance of surviving gut passage and therefore the potential for

1 endozoochorous dispersal (van Leeuwen *et al.*, 2023). However, it is more challenging to
2 quantify and consequently studied less frequently (Dayrell *et al.*, 2023; Carta *et al.*, 2024).

3 Seed mass and shape have been repeatedly hypothesised to be related to seed persistence,
4 with somewhat varying results. A connection between a persistent seed bank and small,
5 spherical seeds has already been noted by Thompson in 1987. Subsequently, clear evidence
6 for the correlation between the size, shape, and persistence of seeds has been
7 demonstrated in the British flora by Thompson *et al.* (1993): all seeds were found to be
8 persistent within a range defined by a maximum in seed mass and seed shape variance.
9 Since then, this relationship has been studied in the flora of other regions as well. Several
10 such studies have confirmed that persistent seeds tend to be smaller and more spherical
11 than transient seeds in various floras, for example in Sweden (Bakker *et al.*, 1996), Argentina
12 (Funes *et al.*, 1999), Iran (Thompson *et al.*, 2001), Italy (Cerabolini *et al.*, 2003), and China
13 (Zhao *et al.*, 2011). A recent study synthesising available data for 1,474 species worldwide
14 also found that persistent seeds tend to be small and spherical (Wang *et al.*, 2024). On the
15 other hand, Peco *et al.* (2003), for example, found that although persistent seeds tend to be
16 smaller, seed shape is not related to persistence in Mediterranean grasslands and
17 scrublands in Spain. Conversely, McDonald *et al.* (1996) found that while persistent seeds
18 did tend to be more spherical, seed weight was not related to persistence in a flood meadow
19 in Great Britain. To complicate things even further, no relationship was detected between
20 seed size, seed shape and persistence in some other regions, such as Australia (Leishman
21 and Westoby, 1998) or South Africa (Holmes and Newton, 2004).

22 The relationship between seed traits and seed persistence appears to be highly context-
23 dependent and varies across different floras worldwide. Both factors can be influenced by a
24 range of environmental conditions (e.g., Harel *et al.*, 2011; Abedi *et al.*, 2014; Chen *et al.*,
25 2021), while regional natural history and disturbance regimes further shape how variations
26 in seed traits translate into differences in seed persistence (see e.g., Leishman and Westoby,
27 1998). Given these complexities, it is essential to assess the relationship between seed traits
28 and seed persistence in various regions of the world. Expanding analyses to floras with
29 varying climates and evolutionary histories will provide a more comprehensive

1 understanding of the strength and generality of these relationships (Leishman and Westoby,
2 1998).

3 To our knowledge, how seed size and shape are related to seed persistence has never been
4 studied in the Central European flora, presumably due to the scarcity of seed shape data for
5 the species of Central Europe. To facilitate the analysis of the relationship between these
6 factors, we set out to characterise the seed shape of a large number of plant species of the
7 Pannonian Biogeographical Region, which is located in the eastern part of Central Europe
8 and is bordered by the Carpathians, the Alps, and the Dinaric Mountains (EEA, 2016). We
9 collected regional data on the seed mass and seed persistence of 392 species of the
10 Pannonian flora and quantified the seed shape of all species. By analysing the compiled
11 dataset, we aimed to answer the following questions: i) How seed mass and seed shape are
12 related to seed persistence? ii) Is this relationship consistent across plant functional
13 groups? and iii) Are seed mass and shape related to each other and is this relationship
14 consistent across plant functional groups?

15

16 **MATERIALS AND METHODS**

17

18 **Data collection**

19 To analyse the relationship of seed traits and seed persistence in the Pannonian flora, we
20 collated a dataset fully based on regionally measured data, in order to avoid the potential
21 confounding effects of distinct climates which can cause considerable intraspecific trait
22 variability (Albert *et al.*, 2010; Sonkoly and Török, 2024). The Pannonian Database of Plant
23 Traits (PADAPT, Sonkoly *et al.*, 2023) contains seed persistence data for more than 600
24 species based on regional soil seed bank studies. From these, we selected those species
25 which are included in the seed collection of the Department of Ecology, University of
26 Debrecen, and therefore available for seed shape measurements (424 species in total). In
27 PADAPT, data on seed persistence is provided in the form of Seed Bank Persistence Index
28 (SBPI) following the approach of the longevity index by Bekker *et al.* (1998). SBPI represents

1 the proportion of data indicating the presence of a persistent seed bank for a species,
2 ranging from zero to one. SBPI = 0 indicates that all available data suggest a transient seed
3 bank for the species, while SBPI = 1 indicates that all available data suggest a persistent seed
4 bank for the species. The thousand-seed mass (TSM) of the 424 species have already been
5 measured on seeds stored in the aforementioned seed collection (see Török *et al.*, 2013,
6 2016; Törő-Szjgyártó *et al.*, 2023).

7

8 **Seed shape measurements**

9 To quantify seed shape, we measured the width, length and thickness of the seeds of all 423
10 species. Twenty replicate measurements were performed for each species and then all
11 width, length and thickness data were averaged between the 20 measurements. Thickness
12 was measured using a HEDÜ 510-201 digital thickness gauge, with an accuracy of 0.02 mm.
13 The length and width values of the same 20 seeds were obtained from photographs using
14 WinMag 1.0 data acquisition system. In general, we aimed to measure diaspores in the form
15 they are dispersed, meaning that the measured morphological unit was not necessarily a
16 seed for all species, but here we refer to all of them as seeds for simplicity. Most grass and
17 Asteraceae seeds were measured without appendage. *Rumex* seeds were also measured
18 without appendage, as the presence of appendages makes accurate measurements
19 difficult. The measured morphological unit for each species is given in Supplementary Table
20 S1. The seed shape measurements were carried out on the same seed lots which were
21 previously used for thousand-seed mass measurements (see Török *et al.*, 2013, 2016; Törő-
22 Szjgyártó *et al.*, 2023), ensuring that the measured morphological units were the same for
23 seed mass and shape measurements.

24

25 **Data analysis**

26 To reduce the number of confounding factors, we excluded two species groups from the
27 analyses. We excluded aquatic plants because seed bank formation and seed persistence
28 in aquatic habitats is presumably influenced differently by seed traits compared to terrestrial
29 habitats. Following the approach of Powney *et al.* (2014), we categorised species with a soil

1 moisture indicator value above 8 as aquatic (based on Borhidi, 1995) and excluded eight
2 species from the analyses based on this criterium. Trees and shrubs were also excluded
3 from the analyses as their seed persistence seems to be influenced by seed traits differently
4 than that of herbaceous species (see Wang *et al.*, 2024) and they were represented by too
5 few species to be analysed separately. We categorised the species into life forms based on
6 Sonkoly *et al.* (2023) and all phanaerophyte species including nanophanaerophytes
7 (subshrubs), microphanerophytes (shrubs), and mega-mesophanerophytes (trees),
8 altogether 24 species, were excluded from the analyses. After these exclusions, our dataset
9 contained 392 species (see Table S1).

10 As a measure of seed shape, we calculated the Seed Shape Index for each species. Seed
11 Shape Index expresses how much the shape of a seed differs from being spherical, with a
12 value of zero indicating a perfectly spherical seed. Increasing Seed Shape Index values
13 indicate increasingly flattened and/or elongated seeds. For needle- or disc-shaped seeds,
14 the maximum value is about 0.3 and varies very little between seeds of the same species.
15 Following the calculations of Thompson *et al.* (1993), we calculated Seed Shape Index as the
16 variance of seed length, width, and thickness. To prevent seed size from affecting the index,
17 we first standardised the three dimensions by scaling them relative to seed length, which
18 was set to 1.

19 To study the association of TSM and Seed Shape Index with seed persistence, we used seed
20 persistence as a binary dependent variable (transient vs. persistent). Following the approach
21 of Gioria *et al.* (2020) and Wang *et al.* (2024) for example, Seed Bank Persistence Index (SBPI)
22 values of zero were treated as having transient seeds, while species with an SBPI higher than
23 zero were treated as having persistent seeds, because a SBPI higher than zero indicates that
24 there was at least one study from Hungary finding a persistent seed bank for the species in
25 question.

26 To correct for the phylogenetic autocorrelation in our data, we used phylogenetic
27 comparative methods. We used the Daphne phylogenetic tree, which is a dated, ultrametric
28 tree of Central European vascular plants encompassing 4685 species (Durka and Michalski,
29 2012). 96.5% of the species in our dataset were represented on the Daphne tree; therefore,

1 for the phylogenetically informed analyses we reduced our dataset to these 368 species and
2 pruned the tree accordingly. The Daphne tree contains polytomies, which we resolved into a
3 binary (bifurcating) tree using the function *multi2di* from the R package *ape* (Paradis *et al.*
4 2004).

5 To analyse the relationship of TSM, Seed Shape Index, and their interaction with seed
6 persistence as a binary variable (persistent vs. transient), we applied phylogenetic logistic
7 regressions (Ives and Garland, 2010) with the function *phyloglm* provided in the R package
8 *phylolm* (Ho and Ane, 2014). We used the 'logistic_MPLE' method with 1000 independent
9 bootstrap replicates. *Phyloglm* uses α to quantify the phylogenetic signal, with smaller α
10 values indicating a stronger phylogenetic signal. We also applied standard logistic
11 regressions and compared the standard and phylogenetic models based on the Akaike
12 Information Criterion (AIC). Because TSM and Seed Shape Index are on very different scales
13 (TSM ranged from 0.004 g to 56.14 g, while Seed Shape Index ranged from 0.00013 to
14 0.29329), we used z-scoring prior to the analysis to standardise them so that both variables
15 have a mean of zero and a standard deviation of one, which also makes the coefficients and
16 odds ratios more comparable.

17 We also used phylogenetic logistic regressions to analyse the relationship of TSM and Seed
18 Shape Index with seed persistence in four plant functional groups defined by two
19 complementary classification aspects: growth form (forbs vs. graminoids) and life span
20 (perennial vs. short-lived). Thus, the first two groups (forbs and graminoids) and the latter
21 two groups (perennials and short-lived species) refer to the same set of species but
22 classified according to different criteria. Life form was assigned to species according to
23 Sonkoly *et al.* (2023) and we considered therophyte and hemitherophyte species to be short-
24 lived (129 species after reducing our dataset to species also included in the Daphne tree).
25 Species in other life form categories were considered perennial (239 species). Species in the
26 Cyperaceae, Juncaceae and Poaceae families were considered graminoids (87 species), all
27 other herbaceous species were considered to be forbs (283 species). We compared the TSM
28 and the Seed Shape Index of persistent vs. transient species using the function *phylANOVA*
29 in the *phytools* package (Revell, 2024), which performs a simulation-based phylogenetic

1 ANOVA (Garland *et al.* 1993). As some p-values were close to the 0.05 significance
2 threshold, we used 10,000 simulations (nsim=10000) to ensure sufficient precision.

3 To test how TSM and Seed Shape Index are related to each other across all species and in
4 different functional groups, we performed phylogenetic generalized least squares (PGLS)
5 regressions with TSM as dependent and Seed Shape Index as explanatory variable. Based on
6 the recommendation of Chen *et al.* (2023), we calculated Pagel's λ (a widely used
7 phylogenetic signal metric, Pagel 1999) for both traits and used the one with a higher Pagel's
8 λ as the dependent variable. We implemented PGLS regressions using the R packages *ape*
9 (Paradis *et al.*, 2004) and *nlme* (Pinheiro *et al.*, 2014). Pagel's λ was calculated using the
10 *phylosig* function in the *phytools* R package (Revell, 2024). All analyses were carried out in
11 an R environment (version 4.3.2, R Core Team, 2023). Nomenclature follows Euro+Med
12 PlantBase (<http://www.europlusmed.org>).

14 RESULTS

15 After the exclusion of aquatic and woody species, and reducing our dataset to species also
16 included in the Daphne tree, we performed the analyses with a dataset containing data on
17 368 species (see Table S1). In this reduced dataset, TSM ranged from 0.004 g (*Gnaphalium*
18 *uliginosum* and *Sagina procumbens*) to 56.14 g (*Iris pseudacorus*) while Seed Shape Index
19 ranged from 0.00013 (*Vicia angustifolia*) to 0.29329 (*Stipa borysthenica*).

20 Across all the species, the phylogenetic logistic regression model estimated a low
21 phylogenetic correlation parameter ($\alpha=0.095$), indicating a strong phylogenetic signal in
22 seed persistence, corroborating that correcting for phylogenetic non-independence in the
23 model was necessary. Accounting for phylogenetic non-independence, we found that both
24 TSM and Seed Shape Index were significantly negatively related to seed persistence, with
25 TSM having a stronger relationship than Seed Shape Index (Table 1). The interaction term was
26 also significant, indicating that at higher Seed Shape Index values the relationship of TSM
27 and persistence becomes less negative (see Fig. 1). The threshold TSM was found to be
28 0.054 g, meaning that all studied species with a thousand-seed mass lower than this had a

1 persistent seed bank (Fig. 1). Model comparison based on Akaike Information Criterion (AIC)
2 also indicated that the phylogenetic logistic regression provided a better fit than the
3 standard logistic regression ($\Delta AIC = 3.476$, $AIC = 375.06$ and $AIC = 378.53$, respectively),
4 validating the use of phylogenetic correction in the analysis.

5 By analysing the seed mass of species using phylogenetic ANOVA, we found that the seed
6 mass of species with transient seeds was significantly higher than that of species with
7 persistent seeds after correcting for phylogenetic non-independence ($F = 24.226$, $p = 0.0016$,
8 Fig. 2). However, phylogenetic ANOVA did not show a significant difference between the
9 Seed Shape Index of species with transient and persistent seeds ($F = 10.384$, $p = 0.0557$).

10 To assess whether the effect of TSM and Seed Shape Index on seed persistence is consistent
11 across functional groups, we also studied the relationship separately in four functional
12 groups (Fig. 3). In the case of forb species, the phylogenetic logistic regression model
13 estimated a low phylogenetic correlation parameter ($\alpha = 0.039$), indicating a strong
14 phylogenetic signal in seed persistence in forb species as well. The model indicated that in
15 forb species, both TSM, Seed Shape Index and their interaction had a significant negative
16 association with seed persistence after correcting for phylogenetic non-independence
17 (Table 2). The phylogenetic logistic regression model for forb species provided a slightly
18 better fit to the data than the standard logistic regression ($\Delta AIC = 0.68$, $AIC = 280.24$ and
19 $AIC = 280.92$, respectively). Phylogenetic ANOVA indicated that the seed mass of species
20 with transient seeds was significantly higher than the seed mass of species with persistent
21 seeds in the forb functional group as well ($F = 24.870$, $p = 0.0008$), but the Seed Shape Index
22 did not significantly differ between the two groups in the case of forb species ($F = 1.365385$,
23 $p = 0.466$, Fig. S1).

24 In the case of graminoid species, the phylogenetic logistic regression model also estimated
25 a low phylogenetic correlation parameter ($\alpha = 0.011$), therefore a strong phylogenetic signal
26 in seed persistence. The model indicated that in graminoid species only Seed Shape Index
27 was significantly associated with seed persistence (Table 2). In this case, using a
28 phylogenetic logistic regression did not result in better model fit compared to a standard
29 logistic regression ($\Delta AIC = 3.8501$, $AIC = 105.957$ and $AIC = 102.106$, respectively). In line with

1 the results of the phylogenetic logistic regression, phylogenetic ANOVA indicated that in
2 graminoid species seed mass was not significantly different between species with transient
3 seeds and species with persistent seeds ($F=1.906$, $p=0.547$), while the Seed Shape Index of
4 species with transient seeds was significantly higher than that of species with persistent
5 seeds ($F=14.095$, $p=0.016$, Fig. S1),

6 The phylogenetic logistic regression model estimated a low phylogenetic correlation
7 parameter ($\alpha=0.077$) in the case of perennial species as well, therefore a strong
8 phylogenetic signal in seed persistence. In perennial species only seed mass was
9 significantly associated with seed persistence (Table 2). The phylogenetic logistic regression
10 model for perennial species provided a better fit to the data compared to the standard
11 logistic regression ($\Delta AIC=4.609$, $AIC=329.893$ and $AIC=334.502$, respectively). The
12 phylogenetic ANOVA indicated that in perennial species the seed mass of species with
13 transient seeds was significantly higher than that of species with persistent seeds
14 ($F=16.063$, $p=0.002$), while Seed Shape Index did not significantly differ between the two
15 groups ($F=2.797$, $p=0.193$, Fig. S1), which is also in line with the results of the phylogenetic
16 logistic regression.

17 In the case of short-lived species, the phylogenetic logistic regression model also estimated
18 a low phylogenetic correlation parameter ($\alpha=0.060$), indicating a strong phylogenetic signal
19 in seed persistence in this functional group as well. In the subset of short-lived species, both
20 seed mass, Seed Shape Index, and their interaction were significantly negatively associated
21 with seed persistence (Table 2). In this case, using a phylogenetic logistic regression model
22 provided a slightly better model fit than the standard logistic regression ($\Delta AIC=0.219$,
23 $AIC=69.489$ and $AIC=69.708$, respectively). The phylogenetic ANOVA indicated that in the
24 short-lived group species with transient seeds had a significantly higher seed mass than
25 species with persistent seeds ($F=22.178$, $p=0.0003$), but Seed Shape Index did not
26 significantly differ between the two groups ($F=5.526$, $p=0.093$, Fig. S1).

27 To test whether TSM and Seed Shape Index are related to each other, we used PGLS
28 regression models. Across all species, we found a very weak negative relationship between
29 TSM and Seed Shape Index after accounting for phylogenetic non-independence (Fig. 3A).

1 Similarly weak but significant negative relationships were found between these two
2 variables in the forb and short-lived functional groups (Fig. 3B and 3E), while there was no
3 significant relationship in the case of graminoid species (Fig. 3D) and perennial species (Fig.
4 3D).

5

6 **DISCUSSION**

7 In line with the findings of several previous studies (e.g., Thompson *et al.*, 1993; Funes *et al.*,
8 1999; Zhao *et al.*, 2011), accounting for phylogenetic non-independence, we found that
9 although the relationships are relatively weak, both seed mass and seed shape are
10 significantly negatively related to seed persistence in 368 herbaceous species of the
11 Pannonian flora. However, seed shape appears to be less closely associated with seed
12 persistence in this region. Several previous studies have found that only seed size is
13 significantly related to seed persistence in various regions of the world (e.g., Bekker *et al.*,
14 1998; Peco *et al.*, 2003; Yu *et al.*, 2007; Wang *et al.*, 2011). Therefore, it seems to be a rather
15 common trend that seed shape is less important than seed size in determining seed
16 persistence. However, there are also results implying that only seed shape significantly
17 affects seed persistence (McDonald *et al.*, 1996).

18 Seeds with a thousand-seed mass below 0.054 g were all persistent, implying that in the
19 Pannonian flora all seeds with a mass below this threshold value may be considered
20 persistent with a reasonable certainty. However, many persistent seeds were relatively large,
21 therefore, an upper threshold, above which all seeds could be considered transient, cannot
22 be identified. Similarly, although spherical seeds were found to be more likely to be
23 persistent, many persistent seeds were markedly non-spherical, in line with the findings of
24 Moles *et al.* (2000).

25 Our results agree with the notion that seed persistence cannot be reliably predicted based
26 only on seed mass (Gioria *et al.*, 2020), seed shape or perhaps other seed characteristics
27 such as seed coat thickness, dormancy mechanisms or nutrient reserves may also need to
28 be considered (e.g., Davis *et al.*, 2016; Zalamea *et al.*, 2018). Using seed bank persistence

1 data provided by Thompson *et al.* (1997) and seed mass data measured in the Pannonian
2 region, Csontos and Tamás (2003) demonstrated that the proportion of transient species is
3 increasing with increasing seed mass in the Pannonian flora. This indicates that the
4 relationship between seed size and persistence is the same in the Pannonian flora as in most
5 other regions where it was studied, but seed shape has not been considered in this analysis.
6 Moreover, the analysed seed bank persistence data originated from different regions, which
7 may have a confounding effect.

8 Although Wang *et al.* (2024) found no interaction between seed mass and seed shape, the
9 interaction between them was significant in our analysis encompassing all species.
10 However, in contrast to the analysis of Wang *et al.* (2024), our analysis only included
11 herbaceous non-aquatic species. The negative interaction term indicated that at higher
12 Seed Shape Index values the effect of TSM on persistence is less negative, which may be the
13 result of the fact that above a Seed Shape Index of approximately 0.2, there were no species
14 with very small seeds in the dataset. This negative interaction may not exist in floras
15 containing several species with very small, non-spherical seeds.

16 Seed mass and shape may be related to seed persistence due to a number of reasons. The
17 size and shape of seeds presumably affect their ability to move towards deeper soil layers
18 (e.g., Bekker *et al.*, 1998; Schmiede *et al.*, 2009). For example, there are studies suggesting
19 that large and elongated seeds are less likely to be buried by the activity of soil biota
20 (Thompson *et al.*, 1994; Bernhardt, 1995). One theory is that the correlation may be due to
21 this tendency of small and isodiametric seeds to quickly become buried in the soil, because
22 being able to persist until a disturbance brings them to the soil surface again may commonly
23 be necessary for these seeds (Moles *et al.*, 2000). As seeds experience higher rates of
24 predation on the soil surface compared to when they are buried (Hulme, 1998; Jacob *et al.*,
25 2006), it can also be hypothesised that large and elongated or flattened seeds with slow soil
26 penetration cannot escape predation by quickly being buried in the soil (Hulme and Borelli,
27 1999). Therefore, for these species it may be less advantageous to build a persistent seed
28 bank. Buried seeds are also less exposed to germination-stimulating temperature
29 fluctuations and light (Fenner and Thompson, 2005). Larger seeds are generally able to

1 germinate from deeper soil layers (Grundy *et al.*, 2003; Sonkoly *et al.*, 2020), while soil burial
2 typically hinders the germination of small seeds, because they are more likely to have a light
3 requirement for germination (Milberg *et al.*, 2000). This means that they are more likely to
4 remain ungerminated once they are buried, providing them an opportunity to persist in the
5 soil.

6 Whether the relationship between seed persistence and the size and shape of seeds varies
7 between different plant functional groups has also not been studied previously. For example,
8 it is known that to ensure survival during periods of unfavourable environmental conditions,
9 short-lived species more strongly depend on persistent seeds than perennial species (Meyer
10 *et al.*, 2006; Scott *et al.*, 2010). Accordingly, short-lived plant species tend to have more
11 persistent seeds than perennials (Gioria *et al.*, 2020), which are typically also smaller-sized
12 than the seeds of perennial species (Thompson *et al.*, 1998; Wang *et al.*, 2011). A short life-
13 span can also be associated with persistent seeds through the disturbance regime of the
14 habitat. The proportion of short-lived species is higher in disturbed habitats than in relatively
15 undisturbed ones, as disturbance can lead to changes in plant community composition in
16 favour of species with rapid growth and with a resource-acquisitive strategy (Smith *et al.*,
17 2022). As ensuring the survival of the population in disturbed habitats requires the formation
18 of a persistent seed bank (Fenner and Thompson, 2005), the relationship between seed traits
19 and seed persistence may not be the same in short-lived and perennial species. In this
20 context, it has already been demonstrated that different plant functional groups such as
21 annuals and perennials can have contrasting relationships between seed size and several
22 other factors like competitive ability and seed production (Coomes and Grubb, 2003). Seed
23 bank types can also be contrasting in forb and graminoid species even within the same
24 habitat type (Bertiller and Aiola, 1997), and the relationship between habitat characteristics
25 and the proportion of species with persistent seeds can differ significantly in forb and
26 graminoid species (Zeiter *et al.*, 2013). Moreover, the ability of a species' seeds to persist is
27 also related to phylogeny (Gioria *et al.*, 2020).

28 Based on the above considerations, the relationship between seed traits and seed
29 persistence may vary considerably across different plant functional groups, and our findings

1 confirm this assumption. Accounting for phylogenetic non-independence, we found that
2 both seed mass and seed shape were associated with seed persistence across all species
3 and in the forb, and short-lived groups, with seed mass having a stronger relationship with
4 seed persistence in all the above groups. In contrast to this, only seed shape was related to
5 seed persistence in the case of graminoid species, and only seed mass in the case of the
6 perennial group. Although the relationships are relatively weak, these results suggest that
7 there are different effects at play in forb and in graminoid species. Wang *et al.* (2024) found
8 that the relationship of seed mass and shape with seed persistence is not consistent across
9 phylogenetic clades. According to their findings, both seed mass and shape affect
10 persistence in Poales, but only seed mass affects persistence in Asterales and Lamiales,
11 while no significant effect was detected in Fabales and Caryophyllales. Although the nature
12 of the relationship they found in Poales is not the same as what we found in the graminoid
13 group (which consisted mainly of Poales species), their findings are consistent with our
14 results in the sense that the relationship between seed traits and seed persistence in
15 graminoids is different from the relationship seen in other species. Moreover, the
16 relationship of these variables may also differ between short-lived and perennial graminoid
17 species. Taken together, these findings suggest that graminoids exhibit distinct relationships
18 and might have to be treated and analysed separately from other species to disentangle the
19 complex relationships between seed traits and seed bank persistence.

20 If the relationship of seed size and shape with seed persistence is not consistent across
21 different plant functional groups, it may be because they are differently related to each other
22 in different functional groups, which could at least partially explain inconsistencies.
23 However, most previous studies about the influence of seed size and shape on seed
24 persistence have not assessed whether and how seed size and shape themselves are
25 correlated (e.g., Moles *et al.*, 2000; Peco *et al.*, 2003; Wang *et al.*, 2024), leaving this question
26 unresolved. To our knowledge, the study of Zhao *et al.* (2011) is the only exception. They
27 studied 141 species of sand grasslands in Northern China and found a slight tendency of
28 bigger seeds to be more spherical, but the relationship was not significant. We addressed
29 this knowledge gap by examining the relationship between seed size and shape within and

1 across plant functional groups, while also accounting for phylogeny. We found a weak
2 negative correlation between seed mass and Seed Shape Index in all species and in the
3 functional groups of forbs and short-lived species, while, after accounting for phylogeny,
4 there was no significant correlation in graminoid and perennial species. In graminoids, seed
5 shape may counteract or modify the commonly observed effect of seed mass on
6 persistence, making seed mass a non-significant predictor of persistence in this group. In
7 the studied 91 graminoid species of the Pannonian flora, small-seeded species were
8 generally found to have more spherical seeds (e.g., *Juncus* or *Agrostis* species), while larger
9 graminoid seeds are typically more elongated (e.g., *Stipa* or *Bromus* species). Csontos and
10 Kalapos (2013) also found that larger seeds tend to be less isodiametric in 137 grass species
11 of the Pannonian flora with C3 photosynthesis. This trend therefore seems to be quite
12 obvious in the Pannonian flora, but this might not be the case in other regions of the world,
13 which could also cause a different relationship between seed size and shape and seed
14 persistence in the graminoids of other floras. A possible future direction would therefore be
15 to test whether the seed persistence of graminoid species is also influenced solely by seed
16 shape in other floras as well.

17

18 **CONCLUSIONS**

19 Persistent seed banks have a key role in community resilience and in the maintenance of
20 biodiversity through space and time; therefore, enhancing our knowledge of the formation of
21 persistent seed banks is vitally important. Our results contribute to a better understanding
22 of the factors linked to seed persistence in the soil. We found that similarly to the floras of
23 several other regions, both seed size and seed shape are significantly related to seed
24 persistence in the Pannonian flora, with seed shape having a less strong influence. By
25 analysing the relationship between these factors in different plant functional groups
26 separately, we also revealed that graminoid species show distinct relationships. Therefore,
27 although the general trend found in our study is consistent with most previous analyses of
28 seed size, seed shape, and persistence, our more detailed results regarding different plant
29 functional groups suggest that detailed analyses are necessary in the floras of other regions

1 as well and future studies of this relationship may need to treat and analyse graminoid
2 species separately.

3

4

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12

13 **AUTHOR CONTRIBUTIONS**

14 V.T-S: investigation, methodology, data curation, writing–original draft. P.T:
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16 investigation. H.M-R: investigation. L.R.G.S: investigation. S.M: investigation. G.K-V:
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19

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23

24 **DATA AVAILABILITY STATEMENT**

25 All the data generated for and used in this study is available in Supplementary Table S1.

26

1 LITERATURE CITED

- 2 Abedi M, Bartelheimer M, Poschlod P. 2014. Effects of substrate type, moisture and its interactions
3 on soil seed survival of three *Rumex* species. *Plant and Soil* 374: 485–495. DOI: 10.1007/s11104-013-
4 1903-x
- 5 Albert CH, Thuiller W, Yoccoz NG, Douzet R, Aubert S, Lavorel S. 2010. A multi-trait approach reveals
6 the structure and the relative importance of intra-vs. interspecific variability in plant traits. *Functional*
7 *Ecology* 24: 1192–1201. DOI: 10.1111/j.1365-2435.2010.01727.x
- 8 Aparicio A, Albaladejo RG, Ceballos GL. 2002. Genetic differentiation in silicicolous *Echinospartum*
9 (Leguminosae) indicated by allozyme variability. *Plant Systematics and Evolution* 230: 189–201. DOI:
10 10.1007/s006060200004
- 11 Bakker JP, Bakker ES, Rosén E, Verweij GL, Bekker RM. 1996. Soil seed bank composition along a
12 gradient from dry alvar grassland to Juniperus shrubland. *Journal of Vegetation Science* 12: 165–176.
13 DOI: 10.2307/3236316
- 14 Baskin CC, Baskin JM. 2014. *Seeds. Ecology, biogeography and evolution of dormancy and*
15 *germination*. Amsterdam: Elsevier.
- 16 Bekker RM, Bakker JP, Grandin U. *et al.* 1998. Seed size, shape and vertical distribution in the soil:
17 indicators of seed longevity. *Functional Ecology* 12: 834–842. DOI:10.1046/j.1365-
18 2435.1998.00252.x
- 19 Bernhardt K-G. 1995. Seed burial by soil burrowing beetles. *Nordic Journal of Botany* 15: 257–260.
20 DOI: 10.1111/j.1756-1051.1995.tb00151.x
- 21 Bertiller MB, Aloia DA. 1997. Seed bank strategies in Patagonian semi-arid grasslands in relation to
22 their management and conservation. *Biodiversity & Conservation* 6: 639–650. DOI:
23 10.1023/a:1018397615476
- 24 Borhidi A. 1995. Social behaviour types, the naturalness and relative ecological indicator values of
25 the higher plants in the Hungarian flora. *Acta Botanica Hungarica* 39: 97–181.
- 26 Cabin RJ. 1996. Genetic comparisons of seed bank and seedling populations of the desert mustard
27 *Lesquerella fendleri*. *Evolution* 50: 1830–1841. DOI: 10.1111/j.1558-5646.1996.tb03569.x
- 28 Carta A, Vandeloos F, Ramírez-Barahona S. *et al.* 2024. The seed morphospace, a new contribution
29 towards the multidimensional study of angiosperm sexual reproductive biology. *Annals of Botany*
30 134: 701–710. DOI:10.1093/aob/mcae099
- 31 Cerabolini B, Ceriani RM, Caccianiga M, De Andreis R, Raimondi B. 2003. Seed size, shape and
32 persistence in soil: a test on Italian flora from Alps to Mediterranean coasts. *Seed Science Research*
33 13: 75–85. DOI: 10.1079/SSR2002126
- 34 Chambers JC, MacMahon JA, Haefner JH. 1991. Seed entrapment in alpine ecosystems: effects of
35 soil particle size and diaspore morphology. *Ecology* 72: 1668–1677. DOI: 10.2307/1940966
- 36 Chen D, Chen X, Jia C, Wang Y, Yang L, Hu X. 2021. Effects of precipitation and microorganisms on
37 persistence of buried seeds: a case study of 11 species from the Loess Plateau of China. *Plant Soil*
38 467: 181–195. DOI: 10.1007/s11104-021-04990-1

- 1 Chen Z, Guo H, Niu D. 2023. Dependent variable selection in phylogenetic generalized least squares
2 regression analysis under Pagel's lambda model. *bioRxiv preprint*. DOI: 10.1101/2023.05.21.541623
- 3 Coomes DA, Grubb PJ. 2003. Colonization, tolerance, competition and seed-size variation within
4 functional groups. *Trends in Ecology & Evolution* 18: 283–291. DOI: 10.1016/S0169-5347(03)00072-
5 7
- 6 Csontos P, Kalapos T. 2013. More lightweight and isodiametric seeds for C4 than for C3 grasses are
7 associated with preference for open habitats of C4 grasses in a temperate flora. *Grass and Forage
8 Science* 68: 408–417. DOI: 10.1111/gfs.12003
- 9 Csontos P, Tamás J. 2003. Comparisons of soil seed bank classification systems. *Seed Science
10 Research* 13: 101–111. DOI: 10.1079/SSR2003129
- 11 Davis AS, Fu X, Schutte BJ, Berhow MA, Dalling JW. 2016. Interspecific variation in persistence of
12 buried weed seeds follows trade-offs among physiological, chemical, and physical seed defenses.
13 *Ecology and Evolution* 6: 6836–6845. <https://doi.org/10.1002/ece3.2415>
- 14 Dayrell RL, Ott T, Horrocks T, Poschlod P. 2023. Automated extraction of seed morphological traits
15 from images. *Methods in Ecology and Evolution* 14: 1708–1718. DOI: 10.1111/2041-210X.14127
- 16 del Cacho M, Lloret F. 2012. Resilience of Mediterranean shrubland to a severe drought episode: the
17 role of seed bank and seedling emergence. *Plant Biology* 14: 458–466. DOI: 10.1111/j.1438-
18 8677.2011.00523.x
- 19 Durka W, Michalski SG. 2012. Daphne: A dated phylogeny of a large European flora for
20 phylogenetically informed ecological analyses: Ecological Archives E093-214. *Ecology* 93: 2297–
21 2297. DOI:10.1890/12-0743.1
- 22 Estrada A, Meireles C, Morales-Castilla I. *et al.* 2015. Species' intrinsic traits inform their range
23 limitations and vulnerability under environmental change. *Global Ecology and Biogeography* 24: 849–
24 858. DOI: 10.1111/geb.12306
- 25 European Environmental Agency (EEA) 2016. Biogeographical regions. Source:
26 <https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>
- 27 Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge: Cambridge University Press. DOI:
28 10.1017/CBO9780511614101
- 29 Funes G, Basconcelo S, Díaz S, Cabido M. 1999. Seed size and shape are good predictors of seed
30 persistence in soil in temperate mountain grasslands of Argentina. *Seed Science Research* 9: 341–
31 345. DOI: 10.1017/S0960258599000355
- 32 Garland Jr, T, Dickerman AW, Janis CM, Jones JA. 1993. Phylogenetic analysis of covariance by
33 computer simulation. *Systematic Biology* 42: 265–292. DOI: 10.1093/sysbio/42.3.265
- 34 Gioria M, Pyšek P, Baskin CC, Carta A. 2020. Phylogenetic relatedness mediates persistence and
35 density of soil seed banks. *Journal of Ecology* 108: 2121–2131. DOI: 10.1111/1365-2745.13437
- 36 Greuter W, von Raab-Straube E, Raus T. 2024. *Euro+Med PlantBase – the information resource for
37 Euro-Mediterranean plant diversity*. <https://euoplusmed.org/>. Accessed 10 Dec. 2024.

- 1 Grundy AC, Mead A, Burston S. 2003. Modelling the emergence response of weed seeds to burial
2 depth: interactions with seed density, weight and shape. *Journal of Applied Ecology*, 40, 757–770.
3 DOI:10.1046/j.1365-2664.2003.00836.x.
- 4 Harel D, Holzapfel C, Sternberg M. 2011. Seed mass and dormancy of annual plant populations and
5 communities decreases with aridity and rainfall predictability. *Basic and Applied Ecology* 12: 674–
6 684. DOI: 10.1016/j.baae.2011.09.003
- 7 Ho LST, An C. 2014. A linear-time algorithm for Gaussian and non-Gaussian trait evolution models.
8 *Systematic Biology* 63: 397–408. DOI: 10.1093/sysbio/syu005
- 9 Holmes PM, Newton RJ. 2004. Patterns of seed persistence in South African fynbos. *Plant Ecology*
10 172: 143–158. DOI: 10.1023/b:vege.0000026035.73496.34
- 11 Hopfensperger KN. 2007. A review of similarity between seed bank and standing vegetation across
12 ecosystems. *Oikos* 116: 1438–1448. DOI: 10.1111/j.0030-1299.2007.15818.x
- 13 Hulme PE. 1998. Post-dispersal seed predation and seed bank persistence. *Seed Science Research*
14 8: 513–519. DOI: 10.1017/S0960258500004487
- 15 Hulme PE, Borelli T. 1999. Variability in post-dispersal seed predation in deciduous woodland:
16 relative importance of location, seed species, burial and density. *Plant Ecology* 145: 149–156. DOI:
17 10.1023/a:1009821919855
- 18 Ives AR, Garland Jr. T. 2010. Phylogenetic logistic regression for binary dependent variables.
19 *Systematic Biology* 59: 9–26. DOI: 10.1093/sysbio/syp074
- 20 Jacob HA, Minkey DM, Gallagher RS, Borger CP. 2006. Variation in postdispersal weed seed predation
21 in a crop field. *Weed Science* 54: 148–155. DOI: 10.1614/WS-05-075R.1
- 22 Jaganathan GK, Boenisch G, Kattge J, Dalrymple SE. 2019. Physically, physiologically and
23 conceptually hidden: Improving the description and communication of seed persistence. *Flora* 257:
24 151413. DOI: 10.1016/j.flora.2019.05.012
- 25 Kalamees R, Püssa K, Zobel K, Zobel M. 2012. Restoration potential of the persistent soil seed bank
26 in successional calcareous (alvar) grasslands in Estonia. *Applied Vegetation Science* 15: 208–218.
27 DOI: 10.1111/j.1654-109X.2011.01169.x
- 28 Kiss R, Deák B, Török P, Tóthmérész B, Valkó O. 2018. Grassland seed bank and community resilience
29 in a changing climate. *Restoration Ecology* 26: S141–S150. DOI: 10.1111/rec.12694
- 30 Levin D. 1990. The seed bank as a source of genetic novelty in plants. *The American Naturalist* 135:
31 563–572. DOI: 10.1086/285062
- 32 Leishman MR, Westoby M. 1998. Seed size and shape are not related to persistence in soil in
33 Australia in the same way as in Britain. *Functional Ecology* 12: 480–485. DOI: 10.1046/j.1365-
34 2435.1998.00215.x
- 35 Long RL, Gorecki MJ, Renton M. *et al.* 2015. The ecophysiology of seed persistence: A mechanistic
36 view of the journey to germination or demise. *Biological Reviews of the Cambridge Philosophical*
37 *Society* 90: 31–59. DOI: 10.1111/brv.12095

- 1 Meyer SE, Quinney D, Weaver J. 2006. A stochastic population model for *Lepidium papilliferum*
2 (Brassicaceae), a rare desert ephemeral with a persistent seed bank. *American Journal of Botany* 93:
3 891–902. DOI: 10.3732/ajb.93.6.891
- 4 McDonald AW, Bakker JP, Vegelin K. 1996. Seed bank classification and its importance for the
5 restoration of species-rich flood-meadows. *Journal of Vegetation Science* 7: 157–164. DOI:
6 10.2307/3236315
- 7 Milberg P, Andersson L, Thompson K. 2000. Large-seeded species are less dependent on light for
8 germination than small-seeded ones. *Seed Science Research* 10: 99–104. DOI:
9 10.1017/S0960258500000118
- 10 Moles AT. 2018. Being John Harper: Using evolutionary ideas to improve understanding of global
11 patterns in plant traits. *Journal of Ecology* 106: 1–18. DOI: 10.1111/1365-2745.12887
- 12 Moles AT, Hodson DW, Webb CJ. 2000. Seed size and shape and persistence in the soil in the New
13 Zealand flora. *Oikos* 89: 541–545. DOI: 10.1034/j.1600-0706.2000.890313.x
- 14 Moriuchi KS, Venable DL, Pake CE, Lange T. 2000. Direct measurement of the seed bank age structure
15 of a Sonoran Desert annual plant. *Ecology* 81: 1133–1138. DOI: 10.2307/177184
- 16 Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884. DOI:
17 10.1038/44766
- 18 Pakeman RJ, Small JL, Torvell L. 2012. Edaphic factors influence the longevity of seeds in the soil.
19 *Plant Ecology* 213: 57–65. DOI: 10.1007/s11258-011-0006-0
- 20 Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language.
21 *Bioinformatics* 20: 289–290. DOI: 10.1093/bioinformatics/btg412
- 22 Peco B, Traba J, Levassor C, Sánchez AM, Azcárate FM. 2003. Seed size, shape and persistence in dry
23 Mediterranean grass and scrublands. *Seed Science Research* 13: 87–95. DOI: 10.1079/SSR2002127
- 24 Pinheiro J, Bates D, DebRoy S, Sarkar D. 2014. nlme: Linear and Nonlinear Mixed Effects Models. R
25 package version 3.1–117. Available at: <http://cran.r-project.org/web/packages/nlme/index.html>.
- 26 Plue J, De Frenne P, Acharya K. *et al.* 2017. Where does the community start, and where does it end?
27 Including the seed bank to reassess forest herb layer responses to the environment. *Journal of*
28 *Vegetation Science* 28: 424–435. DOI: 10.1111/jvs.12493
- 29 Plue J, Van Calster H, Auestad I. *et al.* 2021. Buffering effects of soil seed banks on plant community
30 composition in response to land use and climate. *Global Ecology and Biogeography* 30: 128–139.
31 DOI: 10.1111/geb.13201
- 32 Powney GD, Rapacciuolo G, Preston CD, Purvis A, Roy DB. 2014. A phylogenetically informed trait-
33 based analysis of range change in the vascular flora of Britain. *Biodiversity and Conservation* 23: 171–
34 185. DOI: 10.1007/s10531-013-0590-5
- 35 R Core Team (2023). R: A language and environment for statistical computing. R Foundation for
36 Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- 37 Revell LJ. 2024. phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and
38 other things). *PeerJ* 12: e16505. DOI: 10.7717/peerj.16505

- 1 Royo AA, Ristau TE. 2013. Stochastic and deterministic processes regulate spatiotemporal variation
2 in seed bank diversity. *Journal of Vegetation Science* 24: 724–734. DOI: 10.1111/jvs.12011
- 3 Ruprecht E, Fenesi A, Fodor EI, Kuhn T, Tökölyi J. 2015. Shape determines fire tolerance of seeds in
4 temperate grasslands that are not prone to fire. *Perspectives in Plant Ecology, Evolution and*
5 *Systematics* 17: 397–404. DOI: 10.1016/j.ppees.2015.07.001
- 6 Saatkamp A, Affre L, Dutoit T, Poschlod P. 2009. The seed bank longevity index revisited: limited
7 reliability evident from a burial experiment and database analyses. *Annals of Botany* 104: 715–724.
8 DOI: 10.1093/aob/mcp148
- 9 Schmiede R, Donath TW, Otte A. 2009. Seed bank development after the restoration of alluvial
10 grassland via transfer of seed-containing plant material. *Biological Conservation* 142: 404–413. DOI:
11 10.1016/j.biocon.2008.11.001
- 12 Scott K, Setterfield S, Douglas M, Andersen A. 2010. Soil seed banks confer resilience to savanna
13 grass-layer plants during seasonal disturbance. *Acta Oecologica* 36: 202–210. DOI:
14 10.1016/j.actao.2009.12.007
- 15 Smith EA, Holden EM, Brown C, Cahill Jr JF. 2022. Disturbance has lasting effects on functional traits
16 and diversity of grassland plant communities. *PeerJ* 10: e13179. DOI: 10.7717/peerj.13179
- 17 Sonkoly J, Valkó O, Balogh N. *et al.* 2020. Germination response of invasive plants to soil burial depth
18 and litter accumulation is species-specific. *Journal of Vegetation Science* 31: 1079–1087. DOI:
19 10.1111/jvs.12891
- 20 Sonkoly J, Tóth E, Balogh N. *et al.* 2023. PADAPT 1.0 – the Pannonian dataset of plant traits. *Scientific*
21 *Data* 10: 742. DOI: 10.1038/s41597-023-02619-9
- 22 Sonkoly J, Török P. 2024. Origin of plant trait data matters: Shared species of Northwestern Europe
23 and the Pannonian Ecoregion have different trait values in the two regions (preprint). *bioRxiv* DOI:
24 10.1101/2024.10.14.618145
- 25 Stöcklin J, Fischer M. 1999. Plants with longer-lived seeds have lower local extinction rates in
26 grassland remnants 1950–1985. *Oecologia* 120: 539–543. DOI: 10.1007/s0044 20050888
- 27 Telewski FW, Zeevaart JA. 2002. The 120-yr period for Dr. Beal's seed viability experiment. *American*
28 *Journal of Botany* 89: 1285–1288. DOI: 10.3732/ajb.89.8.1285
- 29 Thompson K. 1987. Seeds and seed banks. *New Phytologist* 106: 23–34. DOI: 10.1111/j.1469-
30 8137.1987.tb04680.x
- 31 Thompson K, Band SR, Hodgson JG. 1993. Seed size and shape predict persistence in soil. *Functional*
32 *Ecology* 7: 236–241. DOI: 10.2307/2389893
- 33 Thompson K, Green A, Jewels AM. 1994. Seeds in soil and worm casts from a neutral grassland.
34 *Functional Ecology* 8: 29–35. DOI: 10.2307/2390108
- 35 Thompson K, Bakker JP, Bekker RM. 1997. *The soil seed banks of North West Europe: methodology,*
36 *density and longevity.* Cambridge: Cambridge University Press. DOI: 10.1046/j.1469-
37 8137.1997.00745-2.x

- 1 Thompson K, Bakker JP, Bekker RM, Hodgson JG. 1998. Ecological correlates of seed persistence in
2 soil in the north-west European flora. *Journal of Ecology* 86: 163–169. DOI: 10.1046/j.1365-
3 2745.1998.00240.x
- 4 Thompson K, Jalili A, Hodgson JG. *et al.* 2001. Seed size, shape and persistence in the soil in an
5 Iranian flora. *Seed Science Research* 11: 345–355. DOI: 10.1079/SSR200191
- 6 Török P, Miglécz T, Valkó O. *et al.* 2013. New thousand-seed weight records of the Pannonian flora and
7 their application in analysing social behaviour types. *Acta Botanica Hungarica* 55: 429–472. DOI:
8 10.1556/abot.55.2013.3-4.17
- 9 Török P, Tóth E, Tóth K. *et al.* 2016. New measurements of thousand-seed weights of species in the
10 Pannonian flora. *Acta Botanica Hungarica* 58: 187–198. DOI: 10.1556/034.58.2016.1-2.10
- 11 Török P, Kelemen A, Valkó O. *et al.* 2018. Succession in soil seed banks and its implications for
12 restoration of calcareous sand grasslands. *Restoration Ecology* 26: S134–S140. DOI:
13 10.1111/rec.12611
- 14 Törő-Szijgyártó V, Balogh N, Henn T. *et al.* 2023. New thousand-seed weight dataset for plant species
15 of Central Europe. *Data in Brief* 48: 109081. DOI:10.1016/j.dib.2023.109081
- 16 van Leeuwen CHA, Soons MB, Vandionant LGVTI, Green AJ, Bakker ES. 2023. Seed dispersal by
17 waterbirds: a mechanistic understanding by simulating avian digestion. *Ecography* 2023: e06470.
18 DOI:10.1111/ecog.06470
- 19 Venable DL, Brown JS. 1988. The selective interactions of dispersal, dormancy, and seed size as
20 adaptations for reducing risk in variable environments. *The American Naturalist* 131: 360–384. DOI:
21 10.1086/284795
- 22 von Blanckenhagen B, Poschlod P. 2005. Restoration of calcareous grasslands: the role of the soil
23 seed bank and seed dispersal for recolonisation processes. *Biotechnology, Agronomy, Society and*
24 *Environment* 9: 143–149
- 25 Wang N, Jiao JY, Jia YF, Wang DL. 2011. Seed persistence in the soil on eroded slopes in the hilly-
26 gullied Loess Plateau region, China. *Seed Science Research* 21: 295–304. DOI: 10.1111/rec.13169
- 27 Wang X, Ge W, Zhang M. *et al.* 2024. Large and non-spherical seeds are less likely to form a persistent
28 soil seed bank. *Proceedings of the Royal Society B* 291: 20232764. DOI: 10.1098/rspb.2023.2764
- 29 Yu S, Sternberg M, Kutiel P, Chen H. 2007. Seed mass, shape, and persistence in the soil seed bank
30 of Israeli coastal sand dune flora. *Evolutionary Ecology Research* 9: 325–340.
- 31 Zalamea PC, Dalling JW, Sarmiento C. *et al.* 2018. Dormancy-defense syndromes and tradeoffs
32 between physical and chemical defenses in seeds of pioneer species. *Ecology* 99: 1988–1998.
- 33 Zeiter M, Preukschas J, Stampfli A. 2013. Seed availability in hay meadows: Land-use intensification
34 promotes seed rain but not the persistent seed bank. *Agriculture, Ecosystems & Environment* 171:
35 55–62. DOI: 10.1016/j.agee.2013.03.009
- 36 Zhao LP, Wu GL, Cheng JM. 2011. Seed mass and shape are related to persistence in a sandy soil in
37 northern China. *Seed Science Research* 21: 47–53. DOI: 10.1017/S0960258510000358

38

1 **FIGURE CAPTIONS**

2 **Figure 1.** The relationship between seed mass, seed shape, and seed bank persistence in
 3 392 herbaceous species of the Pannonian flora. The dashed line indicates the thousand-
 4 seed mass value (0.054 g) below which all studies species have persistent seed banks. Note
 5 that the y-axis is on a logarithmic scale.

6 **Figure 2.** The thousand-seed mass (A) and Seed Shape Index (B) of species with transient vs.
 7 persistent seed banks. Different letters above the bars denote significant differences
 8 (phylogenetic ANOVAs). Note that on figure A the y-axis is on a logarithmic scale.

9 **Figure 3.** The relationship between seed mass, seed shape, and seed bank persistence in
 10 different functional groups accounting for phylogeny: A – all species; B – forb species; C –
 11 graminoid species; D – perennial species; E – short-lived species. The relationship between
 12 seed mass and seed shape were tested using phylogenetic generalized least squares (PGLS)
 13 regressions. Red lines are the PGLS regression lines.

14
 15 **TABLES**

16 **Table 1.** Results of the phylogenetic logistic regression model testing the effects of
 17 thousand-seed mass (TSM), Seed Shape Index, and their interaction on the seed bank
 18 persistence of all herbaceous species. TSM and Seed Shape Index values were scaled before
 19 the analysis (z-scoring). Significant differences are marked with italics.

	Coefficient (Estimate)	Odds Ratio (OR)	95% Confidence Interval (OR)	p-value
TSM	-0.649	0.522	-1.097– -0.346	<0.001
Seed Shape Index	-0.487	0.615	-0.751– -0.234	<0.001
TSM × Seed Shape Index	0.455	1.576	0.121–0.782	0.003

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21

1 **Table 2.** Results of phylogenetic logistic regression models testing the effects of thousand-
 2 seed mass (TSM), Seed Shape Index, and their interaction on the seed bank persistence of
 3 species in different functional groups. TSM and Seed Shape Index values were scaled before
 4 the analysis (z-scoring). Significant differences are marked with italics.

	Coefficient	Odds Ratio	95% Confidence	p-value
	(Estimate)	(OR)	Interval (OR)	
Forb species (n=301)				
TSM	-0.726	0.484	-1.276 – -0.299	<i><0.001</i>
Seed Shape Index	-0.424	0.654	-0.727 – -0.076	<i>0.015</i>
TSM × Seed Shape Index	0.461	1.585	0.044 – 0.921	<i>0.005</i>
Graminoid species (n=91)				
TSM	-0.405	0.667	-1.163 – 0.254	0.259
Seed Shape Index	-0.911	0.402	-1.678 – -0.242	<i>0.002</i>
TSM × Seed Shape Index	0.443	1.558	-0.058 – 1.100	0.074
Perennial species (n=256)				
TSM	-0.622	0.537	-1.288 – -0.243	<i>0.012</i>
Seed Shape Index	-0.307	0.736	-0.612 – 0.006	0.056
TSM × Seed Shape Index	-0.413	1.511	-0.024 – 0.920	0.054
Short-lived species (n=136)				
TSM	-0.907	0.404	-1.549 – 0.000	<i>0.007</i>
Seed Shape Index	-0.895	0.409	-1.589 – -0.117	<i>0.015</i>
TSM × Seed Shape Index	0.534	1.706	0.000 – 1.214	<i>0.039</i>

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6

All species

Seed bank persistence ● persistent ▲ transient

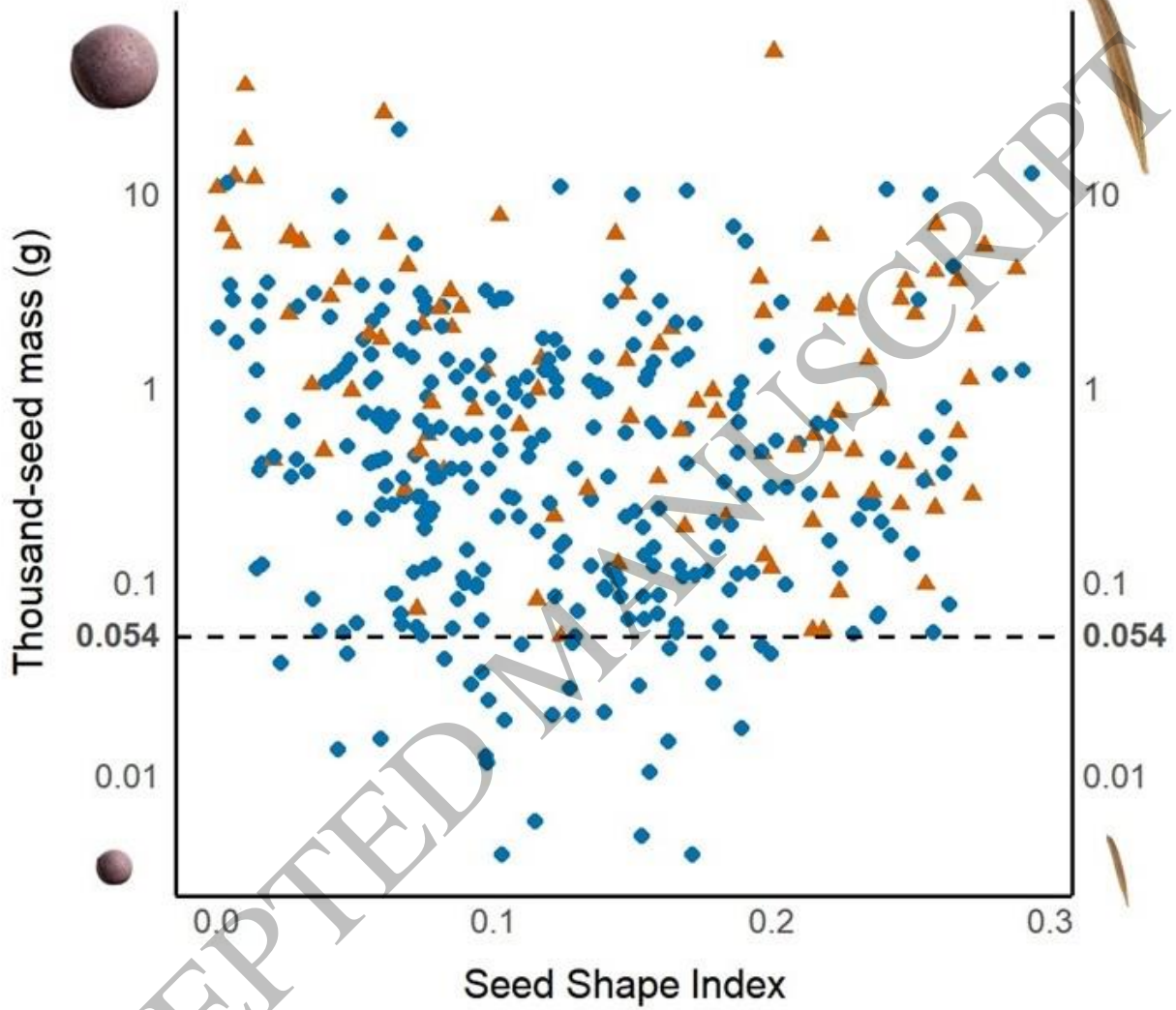


Figure 1
165x160 mm (DPI)

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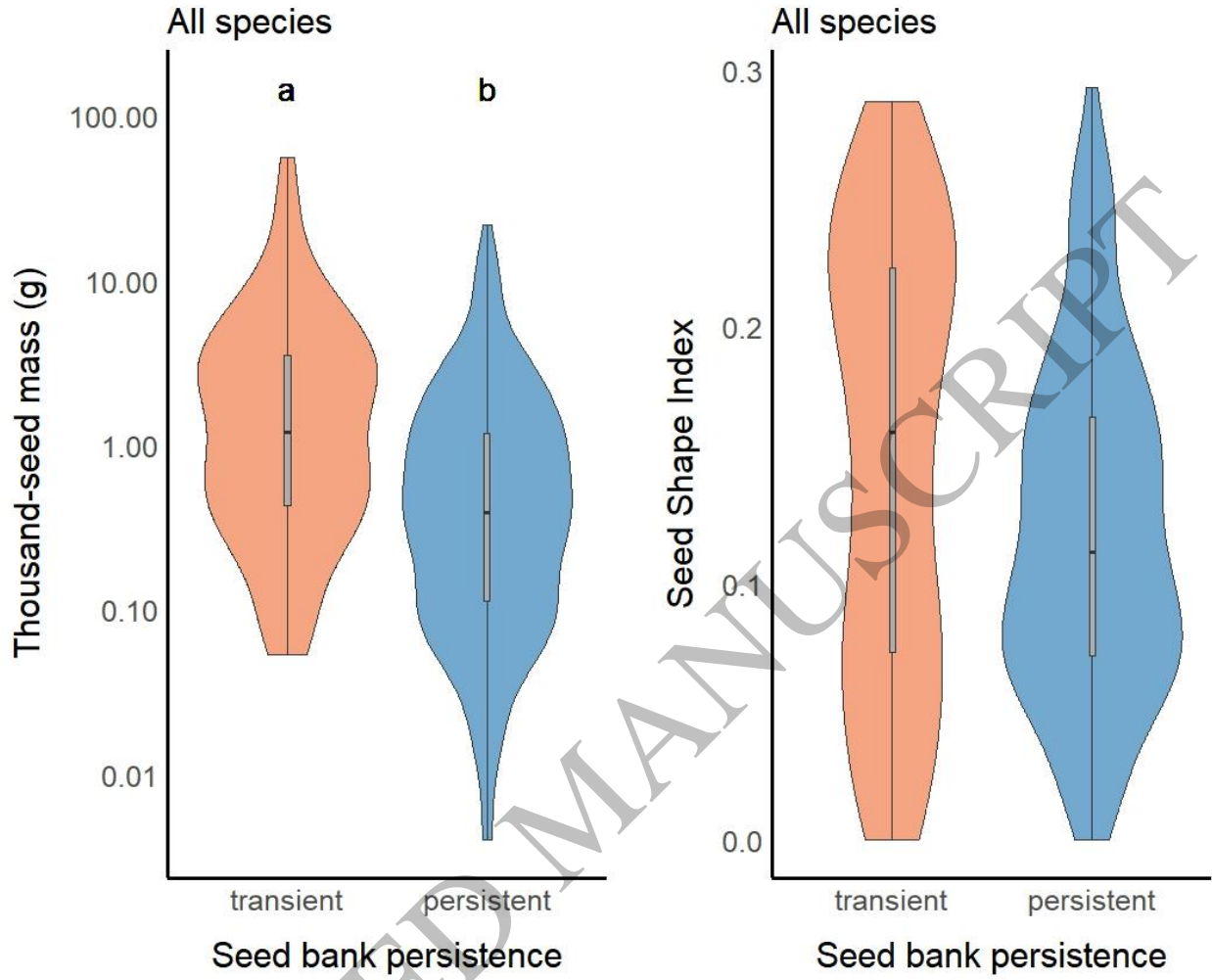
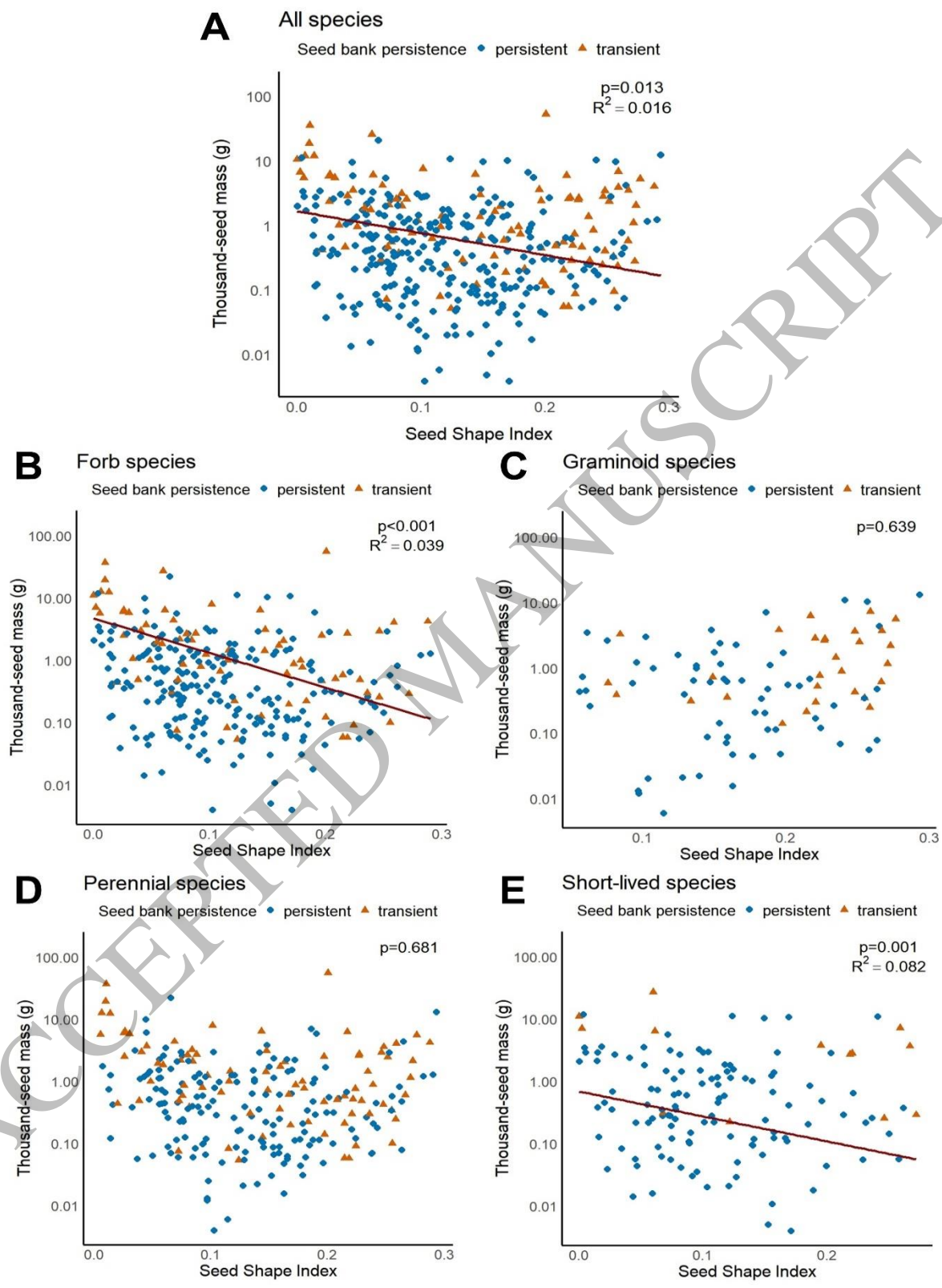


Figure 2
165x134 mm (DPI)

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Figure 3
163x229 mm (DPI)