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Persistence and Changes in Morphological Traits of Herbaceous Seeds Due to Burial in Soil

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Abstract: Seeds in soil banks can survive for many years before conditions become more suitable for germination. Meanwhile, seeds undergo changes in morphology and viability. In this study, we launched an artificial seed bank experiment that included 26 species of seeds. We excavated cohorts for 6–8 consecutive years after burial (*YAB*) in order to determine changes in the morphology (mass, volume, density, seed form) and proportion of fresh (thus persistent) seeds using a crush test as a measure of persistence. The change in seed morphology was fitted by linear and logistic regression, and the proportion of persistent seeds was fitted by logistic regression (effectively by the binomial GLM), which enabled estimation of 50 and 5% persistence times (PT_{50} and PT_{05}). We found that in most species, seed mass, volume and proportion of persistent seeds declined with *YAB*, while other morphological traits were less variable, and the decline in these traits with *YAB* was best fitted with logistic regression. The decline in the proportion of persistent seeds was better fitted by the change in mass than by *YAB* in some species. Among the species included in this study, PT_{50} ranged from 1.2 to 10.5 years, and PT_{05} ranged from 2.1 to 24.3 years. These results can contribute to better understanding of the ecology of weed seed bank persistence in soil. Describing the morphological changes that the seeds undergo in the soil bank may improve our understanding of the biology of seed persistence and facilitate the identification of seeds from the soil bank.

Keywords: seed survival; seed decay; seed persistence in soil; crush test; seed morphology; germination

1. Introduction

The soil seed bank represents a natural storage of plant seeds in soil, in which seeds may persist and remain viable for many years [1]. Depending on the above-ground vegetation dynamics, soil seed banks contain different quantities of seeds. These quantities are largest in arable land, where numbers may exceed 10^5 seeds m^{-2}, and in grasslands (up to 10^4 seeds m^{-2}) [2,3]. Soil seed banks thus provide a "back-up" for future situations when environmental conditions become more suitable for germination (e.g., soil and canopy disturbance) and may prevent local extinction.

Plant species largely differ in the persistence of seed banks. Some species form only a transient seed bank, as seeds that do not germinate quickly do not usually survive more than a year [2,4]. Seeds of other species remain alive for a long time, even hundreds of years [5], and form so-called persistent seed banks [2]. Longevity correlates with seed morphology [6–9], taxonomic affiliation [10,11] and plant ecology [12]; for example, arable species tend to have more persistent seeds compared to forest species [4]. However, within evolutionary constraints of a species, seed persistence is not a fixed trait, and may vary with soil disturbance, type and pH, nutrient content in soil and

(micro-) climatic conditions such as soil moisture and temperature and depth at which the seeds are buried [13–22]. Thus, the realized seed persistence is a combination of seed characteristics and the immediate environment [12].

A variety of approaches have been applied to determine seed persistence in soil [2]. Studies of changes in seed bank composition over time often rely on analyses of series of samples from sites with known time from the last disturbance [5], which is used as a proxy of the minimum seed age. The limitations of these studies for understanding the dynamics of seed persistence in soil lie in the fact that the age determination of the retrieved seeds is very rough and that the initial seed cohort size is unknown. Another common approach includes the creation of artificial seed banks by either sowing the seeds in soil followed by repeatedly taking soil cores and viability testing the retrieved seeds [23,24] or by burying seed bags followed by periodic exhumation and viability testing [2,14,20,25]. With these approaches, the deterioration of the seed population can be followed, parametrized and used for making predictions. Such studies do not usually cover more than 5–6 years [14,21], and those that would span over longer periods of time usually do not collect data on yearly basis [22,26].

In addition to changes in viability, seeds in soil also undergo changes in external morphology [27] caused by soil chemistry and activity of soil microorganisms. There is a lack of literature records that would provide a qualitative description of these changes, which may include changes in colour and loss of trichomes and other extremities or of the seed coat entirely. The little attention paid to these changes may result from the fact that the research has focused mainly on seed persistence per se and that morphological changes have been perceived as too descriptive and thus unimportant.

However, there are several ecological as well as practical reasons to have these changes described for an array of seed species. In the first place, many seeds undergo physical dormancy, i.e., they can germinate only if the seed coat is scarified, i.e., damaged, such as by soil microorganisms or abrasion during movement through soil. Knowing over the course of morphological changes then may become useful for predicting the germinability of the seeds. In the second place, knowledge of the morphological changes can be used in seed identification from soil samples, particularly in species in which these changes are substantial. Knowledge on changes in seed morphology may become relevant in connection with seed predation of exhumed seeds [27–29] because predators are known to select seeds based on seed morphology [30,31]. The aim of this paper is therefore to describe and analyse (i) seed persistence and (ii) changes in morphological seed traits in 26 species of herb seeds in the course of 6–8 years of burial in artificial soil seed banks. Seed persistence was determined over up to 8 years spent in soil, 50 and 5% persistence times were predicted for each seed species based on logistic regression modelling of the proportion of persistent seeds, and changes in morphological traits that can be perceived by seed predators, such as mass, volume, density and form, were examined.

2. Materials and Methods

Seed material. Seeds of 26 species of herbs, mostly weeds, were introduced into artificial seed banks in 2005 and 2006 (Table 1). The species of seeds were selected so they differed in morphology, taxonomic affiliation and presumed persistence in soil, and based on availability. Seed materials were sampled in Prague-Ruzyně (western Czech Republic) in a c. 10 × 10 km area surrounding Crop Research Institute (CRI) and centred at 50.09 N and 14.30 E. The seeds were harvested from mother plants by hand at full ripeness in July-October 2005 and October 2006 and stored dry at 5 °C until the burial. Furthermore, a subset of the seeds was stored at −20 °C as a control cohort (0 years). Seeds of 9 species were buried on 8 November 2005, and the remaining 17 species were buried on 24 October 2006 (Table 1). Seed batches destined for burial for the time period of a particular length (seed cohorts) were prepared in a standard way. Approximately 10^4 seeds were mixed with soil dug from a 0.6 m depth and sieved through 0.05 mm mesh, placed in bags of nylon fabric and buried at a 20 cm depth under grassland on a ground of CRI. Mixing seeds with soil is important to prevent excessive degradation [32], and fine sieving of the soil facilitates separation of the seeds after exhumation. Seed material was divided into 8 (2005) or 6 (2006) batches per species. Each seed batch packed in a bag

with soil was buried separately and connected by a nylon cord with a label on the ground surface. Every year, one batch per seed species was exhumed, and recognizable seeds were separated from soil, dried at 25 °C and 40% r.h., and then stored at −20 °C for experimental use (a cohort). The last batches of seeds buried in 2005 were thus excavated in 2013 after 8 years of burial, and those buried in 2006 were excavated in 2012 after 6 years of burial, so there were 8 or 6 burial cohorts per species, respectively. For *Crepis biennis* 2011 excavation and for *Plantago lanceolata* 2012 excavation, no seeds were available due to complete deterioration of the respective batches.

Table 1. Species of seeds buried in 2005 and 2006 with longevity index (LI) and seed persistence index (SPI) calculated based on the burial data taken from [2]. Number of records indicates how many individual data entries from the database of [3] were used for calculation of the indexes. The nomenclature was based on Kubát et al. [33].

#	Species	Family	Year of Burial	Number of Records	LI	SPI
1	*Amaranthus powellii* S. Watson	*Amaranthaceae*	2006			
2	*Amaranthus retroflexus* L.	*Amaranthaceae*	2006	7	1	3
3	*Atriplex sagittata* Borkh.	*Amaranthaceae*	2006			
4	*Campanula trachelium* L.	*Campanulaceae*	2006			
5	*Capsella bursa-pastoris* (L.) Med.	*Brassicaceae*	2005	20	1	2.55
10	*Chenopodium album* agg.	*Amaranthaceae*	2006	15	1	2.87
11	*Chenopodium glaucum* L.	*Amaranthaceae*	2006			
12	*Chenopodium polyspermum* L.	*Amaranthaceae*	2006			
6	*Crepis biennis* L.	*Asteraceae*	2005			
7	*Geum urbanum* L.	*Rosaceae*	2005			
8	*Hyoscyamus niger* L.	*Solanaceae*	2006			
9	*Hypericum perforatum* L.	*Hypericaceae*	2006	1	1	3
13	*Lavandula angustifolia* Mill.	*Lamiaceae*	2006			
14	*Leonurus cardiaca* L.	*Lamiaceae*	2005			
15	*Lycopus europaeus* L.	*Lamiaceae*	2005			
19	*Persicaria lapathifolia* (L.) Delarbre	*Polygonaceae*	2006	3	1	3
16	*Plantago lanceolata* L.	*Plantaginaceae*	2006	14	0.93	2.43
17	*Plantago major* L.	*Plantaginaceae*	2006	7	0.86	2.57
18	*Plantago media* L.	*Plantaginaceae*	2006	1	1	2
20	*Portulaca oleracea* L.	*Portulacaceae*	2006	6	1	3
21	*Silene noctiflora* L.	*Caryophyllaceae*	2006			
22	*Silene vulgaris* (Moench) Garcke	*Caryophyllaceae*	2006	6	1	3
23	*Thlaspi arvense* L.	*Brassicaceae*	2005	14	1	2.86
24	*Tripleurospermum inodorum* (L.) Schultz-Bip.	*Asteraceae*	2005	18	0.94	2.5
25	*Urtica dioica* L.	*Urticaceae*	2005	1	1	2
26	*Urtica urens* L.	*Urticaceae*	2006	3	1	3

Changes in seed morphology with duration of burial. Prior to measuring changes in seed morphology with duration of burial, subsamples of seeds from each cohort were cleaned from fine soil particles in an ultrasound cleaner (Sonorex RK 31, Bandelin electronic, Berlin, Germany), submerged in water for 2 min, and dried in an oven for 24 hr at 75 °C. The following measurements were made on seeds from each cohort:

Seed mass (M)—For each species and cohort, the average seed mass was determined based on five batches of 20 seeds using analytical balances (CP225D-0CE, Sartorius AG, Göttingen, Germany) with a precision of 0.00001 g.

Seed dimensions—following [19], five seeds per cohort were measured by digital scales NTD12P-15CX (Mitutoyo Corp., Kawasaki, Japan) with a precision of 0.01 mm. The following dimensions were measured: length (L; the longest dimension of the seed), width (W; the longest dimension perpendicular to L within the same plane), and height (H; the longest dimension perpendicular to the plane of L and W). Based on these measurements, the following metrics of the seed form were calculated for each of the five seeds per combination of species and cohort, except for density, which could only be estimated as a mean value:

Volume—calculated as $V = L * W * H$ [34]

Density—calculated as $D = \frac{M}{V}$ [34]; only mean values of mass and volume were used, as these were measured on different seeds.

Shape—according to [19], the seed shape can be expressed as a dimensionless measure: $Vs = \sum \frac{(x-\bar{x})^2}{n}$, where x represents a division of either L, W, and H through L and $\bar{x}$ as their mean, and n is 3. Vs ranges from 0 for perfectly spherical seeds to 0.2 shaped as a thin disc or spindles.

Flatness Index—calculated as $FI = \frac{(L+W)}{2*H}$ [34]. It ranges from 1 for a complete sphere to greater values for plane or spindle like shaped seeds.

Eccentricity Index—calculated as $EI = \frac{L}{W}$ [34]. It ranges from 1 for round seeds to values greater than 2 for spindle like seeds.

Seed persistence in soil. Seed persistence was measured on another subsample of seeds. We used the so-called imbibed seed crush test (ICT), which was found to be reliable for estimation of the true viability of weed seeds [35–37]. This test was performed in order to determine the proportion of fresh [38], i.e., persistent and viable seeds. In these seeds the seed coat does not collapse when crushed with the tips of a pair of forceps, or have apparent and intact cotyledons or embryos when the seed coat is broken. In other cases, the seeds were considered dead. In this test, 20 seeds per cohort tested, and were left imbibe for 3 days in laboratory conditions (20 °C, 12 h light: 12 h dark) prior testing.

Statistical analysis. Prior to analysis, the duration of burial was expressed as a continuous numerical vector, year after burial (*YAB*), and used as the explanatory variable in most of the analyses. Frozen (control) seeds were given age 0, those exhumed after one year were given age 1, etc., so the maximum age was 8 for seeds buried in 2005 and exhumed in 2013 and age 6 for those buried in 2006 and exhumed in 2012. *YAB* 1 then denotes a cohort of seeds that were buried for 1 year and so on. The analyses were performed in R version 3.3.1 [39]. The variation in morphological seed traits with *YAB* was tested within seed species by regression methods [40]. For comparative reasons across species of seeds, the values of M, V and D were converted to relative scale against the mean values of the respective trait for control seeds and denoted rM, rV and rD (i.e., relative mass, relative volume and relative density). The three seed form indexes were not relativized because they are inherently dimensionless. For each species of seeds, the rM, rV and rD were regressed against *YAB* by fitting three different models were used to fit:

(a) Linear: $y = a + b * YAB$, where y is the respective trait on relative scale, a is the intercept, and b is the slope of the linear regression line;

(b) 3-parameter logistic: $y = \frac{A}{1+e^{(D-YAB)/C}}$, where y is the respective trait on relative scale, A is the upper asymptote, C is the scale parameter on the x-axis, and D is an inflexion point of the curve;

(c) 4-parameter logistic: $y = A + \frac{B-A}{1+e^{(D-YAB)/C}}$, where y is the respective trait on relative scale, A is the upper asymptote, B is the lower asymptote, C is the scale parameter on the x-axis, and D is an inflexion point of the curve.

The non-linear curves were fitted as the self-starting functions SSlogis and SSfpl for 3- and 4-parameter logistic functions (nls package of R). The explanatory power of the three models was compared based on the Akaike Information Criterion (AIC) [40], and the one with the lowest AIC value (ΔAIC = 2 as threshold) was chosen as best and presented. Only the linear model was fitted to *Shape*, *FI* and *EI* based on a priori visual data inspection. In case the diagnostic graphs [40] suggested that outlying *YAB* values could influence the parameter estimates, these were removed from model fitting. To better interpret the general pattern in seed form variation with *YAB*, we performed principal component analysis by implementing the *prcomp* function from stats package of R and including rM, rV, rD, *Shape*, *FI* and *EI* as variates, and the resulting principal scores for the first and second axes (*PC1* and *PC2*) were used for interpretation of changes in the proportion of persistent seeds [9].

The variation in the proportion of persistent seeds was assessed based on the logistic regression: $y = \frac{1}{1+e^{-(a+b*x)}}$ [41], and linearized by implementing generalized linear modelling, with a being the intercept and b the slope of the regression, using binomial distribution of errors and logit link function (GLM-b) [17]. This approach takes the nature of the data (proportions of viable seeds in case of

germination test and binary response variable: 0—dead, 1—live into account without a need for transformation [40] and allows for making predictions [17]. The parameter x is either *YAB*, *PC1*, *PC2* and $\overline{rM}$ (i.e., mean relative seed mass per *YAB* cohorts) and the models were compared based on the AIC as above. The seed persistence in the soil was estimated for each species of seed based on the parameters of the model with *YAB*, predicting the 50 and 5% persistence time in *YAB* (PT_{50} and PT_{05}, respectively), i.e., the time after burial when 50 and 5% of the initial seed population were still viable. To identify which morphological traits might have affected the predicted seed persistence across species, we regressed PT_{50} and PT_{05} separately against all traits measured on control seeds against *rM*, *rV* and *rD* for each separate *YAB* cohort and against slopes of change in *Shape*, *FI* and *EI* across *YAB*.

Seed longevity literature survey. Seed longevity data for some of the study species can be excerpted from the literature. The monograph [2] was used as a starting point for the survey. We used only seed persistence data originating from burial experiments (method coded 1–3 after work [2]), which were available for only 14 out of 26 species of seeds included in this study. From the literature data, we calculated two indexes, the longevity index (*LI*) and seed persistence index (*SPI*). *LI* was calculated as: $LI = \frac{R_{sp}+R_{lp}}{R_t+R_{sp}+R_{lp}}$, where R_t, R_{sp} and R_{lp} are the proportion of records classifying the species as transient (persisting less than 1 year), the proportion of records classifying the species as short persistent (persistent for more than 1 year but less than 5 years), and the proportion of records classifying the species as long persistent (persistent for more than 5 years), respectively [4]. *SPI* was calculated as: $SPI = \frac{T+2*SP+3*LP}{total\ number\ of\ records}$, where *T*, *SP* and *LP* represent the number of records reporting transient, short persistence and long persistence, respectively [8]. The values of *LI* and *SPI* with the number of literature records used for calculations are listed in Table 1.

3. Results

Seed mass (as *rM*) decreased with *YAB* in 25 out of 26 species of seeds available. The only exception was *H. niger*, the seeds of which did not change their *rM* with *YAB* (Figure 1a). Logistic curves appeared to fit the decline in *rM* better than the simple linear fit in 17 cases, suggesting that in the majority of species, the rate of mass reduction is slow during the first years after burial, then accelerates and finally slows down again. The 4-parameter logistic curve that included a lower asymptote described the data best in 10 cases, and the 3-parameter curve without the lower asymptote curve did so in seven cases. Examples are in Supplementary Materials. Seed volume (as *rV*) declined with *YAB* in 14 species, of which the linear decrease was found in 10 and logistic in four species, respectively (Figure 1b). The surprising observation that relative seed mass or volume increased after burial in some species of seeds (Figure 1a,b) has to be viewed as an artefact of natural variability among the seeds. Seed density (as *rD*) was even less variable with *YAB* in terms of the number of significant changes (in 11 species only), but the changes were both positive (1 case) and negative (10 cases), suggesting that the mechanisms that lie behind the variation in morphological traits over time spent in soil are diverse across species. Three species of seeds showed a significant linear change, and eight species showed a significant logistic course of change in *rD* with *YAB* (Figure 1c).

The interspecific interrelationship of the three morphological traits and temporal dynamics of their change across *YAB* is exemplified in Figure 2 using fitted values from the above models for *YAB 1*, *5* and *8*. After one year in soil, there was little variation in *rM*, *rV* and hence *rD* (Figure 2a). With the course of time spent in soil, it becomes evident that the rate of change in *rM* and *rV* and hence in *rD* becomes more variable across species (Figure 2b,c)—species distributed along the line representing slope = 1 through the origin are those in which both mass and volume decreased in similar rate, species below the line are those in which the volume decreased more rapidly than mass and species above the line are those in which seed mass declined more rapidly than volume.

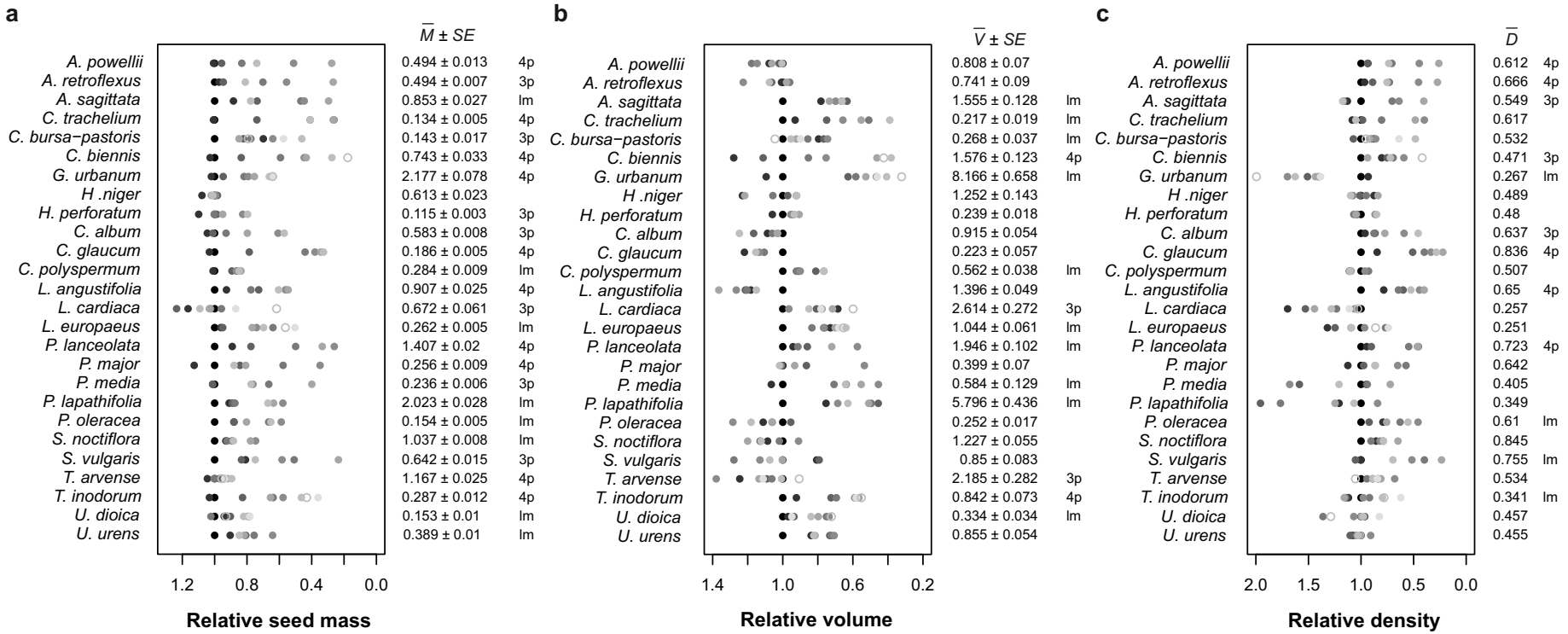

Figure 1. Variation in seed morphology with year after burial (*YAB*) for 26 species of seeds: (**a**) relative seed mass (*rM*); (**b**) seed volume (*rV*); (**c**) relative seed density (*rD*). The decrease in the intensity of grey colour refers to the increase in *YAB*: black = control, white with grey lining = *YAB* 8 are the two extremes). $\overline{M}$ ± SE and $\overline{V}$ ± SE denote the mean dry mass of control seeds ± standard error. $\overline{D}$ denotes the mean dry mass of control seeds. Lm (linear), 3p (3-parameter logistic) and 4p (4-parameter logistic) indicate the model that fitted the change in the seed trait over time based on the Akaike Information Criterion, if any of the three models were significant. See Table 1 for generic names of each species.

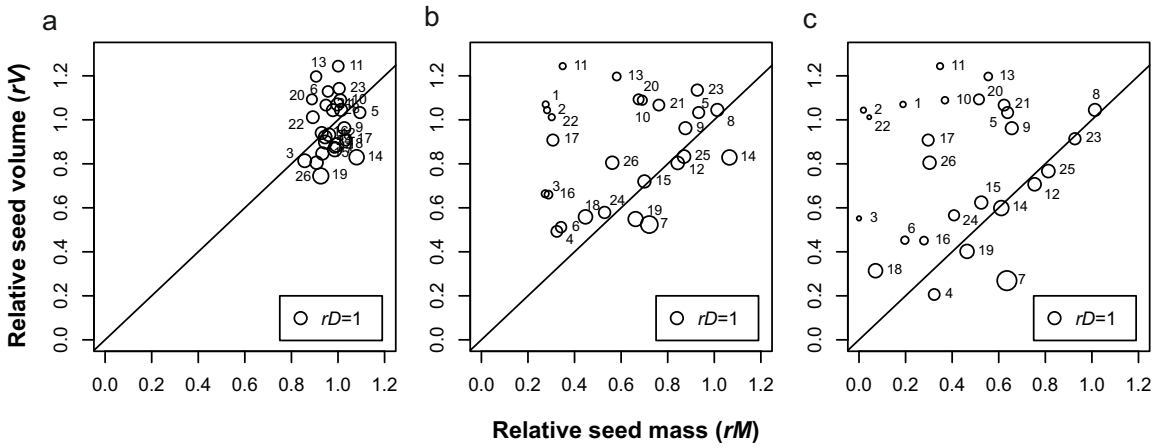

Figure 2. Interrelationship between relative mass (*rM*), volume (*rV*) and density (*rD*) of 26 species of seeds (for species codes, see Table 1). Fitted values from the best models shown in Figures a, b, and c were used, the line of slope = 1 indicates the situation when relative mass and volume declined at the same rate so *rD* did not change. Sizes of the symbols are scaled according to the size of change in *rD*, the symbol size shown on the bottom right equals *rD* = 1, smaller or larger symbols refer to proportional decrease or increase in *rD*, respectively. (**a**) *YAB 1*; (**b**) *YAB 5*; (**c**) *YAB 8.*

The shape of seeds remained similar overall, as the three indexes of the seed form varied with *YAB* in only a few species (Figure 3a–c). Principal component analysis, however, revealed patterns in seed form changes across seed species and *YAB* (Figure 4). The PC1 axis explained 48.4% of the variance and presented the change in external seed form as *Shape*, *FI* and *EI* correlated positively and *rV* negatively with this axis. This suggests that seeds that change their shape also lose their volume as they either shrink and become flatter or lose their extremities. The PC2 axis explained 27.3% of the variance and was well correlated positively with *rM* and *rD*, i.e., represented by seeds that did not change their volume but changed their mass and hence density. The individual variation in all morphological traits with *YAB* for each of the seed species can be found in the Appendix S1: panels A–F.

Alternative models for the proportion of persistent seeds provided mixed results. While the two models based on *PC1* and *PC2* together improved the explanatory power of the variation compared to the ICT model in two cases only (*PC1*: *L. cardiaca*, AIC = 185.28; *PC2*: *C. album*, AIC = 63.86), models based on *rM* explained the change in the proportion of persistent seeds better than did the initial models in 10 cases (Table 2) suggesting that the decline in the proportion of persistent seeds may be associated with the decline in seed mass more tightly than solely to *YAB*, especially when large or inconsistent scatter in *rM* across *YAB* appeared. The change in the proportion of persistent seeds with *YAB* for each of the individual species can be found in the Appendix S1: panels G, and with relative seed mass in the Appendix S1: panels H.

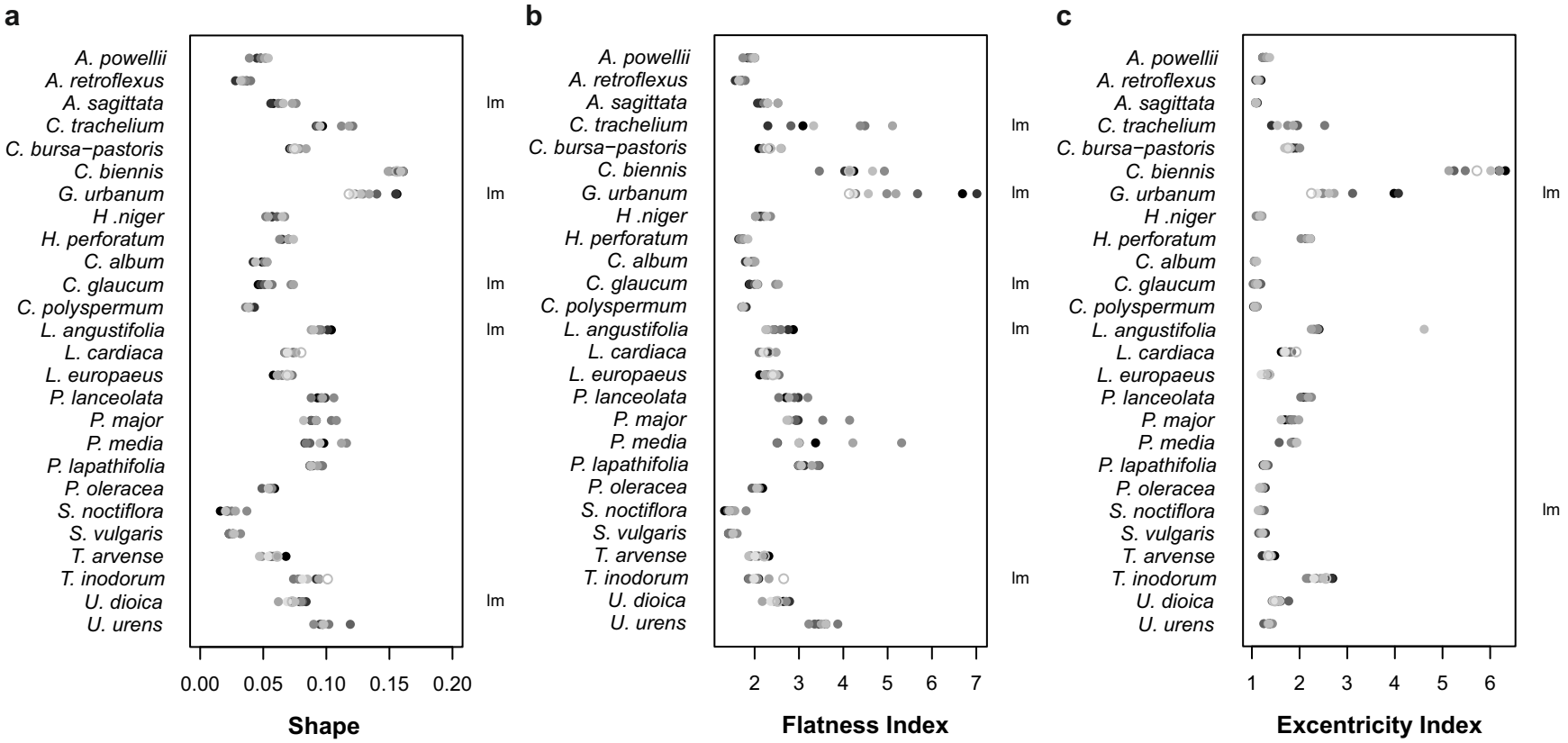

Figure 3. Variation in seed form with year after burial (*YAB*) for 26 species of seeds: (**a**) seed shape; (**b**) flatness index; (**c**) eccentricity index. The value of 0 indicates perfectly spherical seeds, while the value of 0.2 indicates flat or elongated seeds (Figure 3a). The larger the number the flatter (Figure 3b) or eccentric (Figure 3c) the seeds were. The decrease in the intensity of grey colour refers to the increase in *YAB* (i.e., a decrease in the intensity of grey colour): black = control, white with grey lining = *YAB 8* are the two extremes). Lm (linear) indicates the cases when the change in the trait over time was significant according to the linear model. See Table 1 for generic names of each species.

Table 2. Persistence of 26 species of seeds in soil as estimated by logistic regression based on the imbibed crush test (ICT). See Table 1 for the genus of plants. Year after burial (*YAB*) or seed mass relative to control seeds were used as explanatory variable based on 5–8 *YAB* cohorts (N *YAB*). Intercept and slope are estimated parameters *a* and *b*, respectively, of the model: $y = \frac{1}{1+e^{-(a+b*x)}}$, accompanied with 95% confidence intervals (CI), where *y* is the proportion of viable seeds and *x* is *YAB*. AIC—Akaike Information Criterion for the respective model. Significantly better models (ΔAIC>2) are in bold. Persistence times PT_{50} or PT_{05}, accompanied with standard errors (s.e.), show the time in *YAB* when 50 or 5% of the initial cohort of seeds, respectively, were predicted to be still viable.

Species	N *YAB*	*YAB*						Relative Mass	
		Intercept (95% CI)	Slope (95% CI)	P-Value	AIC	Persistence Time ± s.e. [*YAB*]		*P*-Value	AIC
						PT_{50}	PT_{05}		
A. powellii	6	6.578 (4.229–8.926)	−1.963 (−2.641–−1.285)	< 0.001	**70.617**	3.4 ± 0.16	4.9 ± 0.31	< 0.001	102.66
A. retroflexus	6	9.884 (5.372–14.396)	−3.785 (−5.445–−2.125)	< 0.001	**37.37**	2.6 ± 0.12	3.4 ± 0.2	< 0.001	62.65
A. sagittata	6	1.833 (0.891–2.775)	−1.519 (−2.070–−0.968)	< 0.001	**82.18**	1.2 ± 0.19	3.2 ± 0.38	< 0.001	92.14
C. trachelium	6	5.051 (2.838–7.265)	−2.727 (−3.846–−1.609)	< 0.001	**52.30**	1.9 ± 0.14	2.9 ± 0.26	< 0.001	54.77
C. bursa-pastoris	8	4.051 (2.900–5.202)	−0.809 (−1.033–−0.585)	< 0.001	**149.39**	5.0 ± 0.26	8.6 ± 0.60	0.215	243.91
C. album	6	4.938 (3.320–6.557)	−1.130 (−1.505–−0.756)	< 0.001	106.17	4.4 ± 0.22	7.0 ± 0.53	< 0.001	**64.09**
C. glaucum	6	41.940 (-)	−21.663 (-)	< 0.001	24.02	1.9 ± 9.8	2.1 ± 11.05	< 0.001	24.02
C. polyspermum	6	4.537 (2.915–6.159)	−0.791 (−1.133–−0.449)	< 0.001	**105.66**	5.7 ± 0.41	9.5 ± 1.13	< 0.001	115.02
C. biennis	7	2.785 (1.637–3.933)	−1.428 (−1.94–−0.909)	< 0.001	87.49	2.0 ± 0.19	4.0 ± 0.43	< 0.001	86.55
G. urbanum	8	2.448 (1.601–3.295)	−0.238 (−0.393–−0.083)	0.002	174.23	10.3 ± 1.96	22.7 ± 5.96	0.001	172.40
H. niger	6	4.769(2.539–6.998)	−0.513 (−0.976–−0.049)	0.014	**59.25**	9.3 ± 2.32	15.1 ± 4.91	0.537	64.95
H. perforatum	6	2.821 (1.819–3.823)	−0.495 (−0.730–−0.260)	< 0.001	140.85	5.7 ± 0.61	11.6 ± 1 93	< 0.001	**136.22**
L. angustifolia	6	1.595 (0.799–2.392)	−1.000 (−1.334–−0.666)	< 0.001	114.67	2.1 ± 0.16	5.3 ± 0.34	< 0.001	**101.73**
L. cardiaca	8	2.387 (1.600–3.174)	−0.353 (−0.499–−0.207)	< 0.001	198.99	6.8 ± 0.64	15.1 ± 2.23	< 0.001	**189.88**
L. europaeus	8	2.182 (1.360–3.004)	−0.916 (−1.181–−0.652)	< 0.001	132.60	2.4 ± 0.25	5.6 ± 0.50	< 0.001	131.99
P. lanceolata	6	5.099 (2.859–7.340)	−2.192 (−3.158–−1.225)	< 0.001	58.17	2.2 ± 0.18	5.2 ± 0.6	< 0.001	57.60
P. major	5	0.959 (0.312–1.606)	−0.161 (−0.335–−0.013)	0.067	187.35	6.0 ± 1.91	24.3 ± 11.74	< 0.001	**125.59**
P. media	6	2.382 (1.495–3.270)	−0.909 (−1.193–−0.625)	< 0.001	129.86	2.6 ± 0.25	5.9 ± 0.56	< 0.001	**90.92**
P. lapathifolia	6	5.449 (3.595–7.303)	−1.650 (−2.187–−1.114)	< 0.001	82.56	3.3 ± 0.18	5.1 ± 0.35	< 0.001	**73.21**
P. oleracea	6	3.064 (1.988–4.140)	−1.184 (−1.548–−0.820)	< 0.001	**108.33**	2.6 ± 0.21	5.1 ± 0.44	< 0.001	128.00
S. noctiflora	6	3.545 (2.153–4.937)	−0.415 (−0.724–−0.106)	0.004	95.29	8.5 ± 1.79	15.6 ± 4.41	< 0.001	**88.63**
S. vulgaris	6	3.126 (1.790–4.461)	−2.025 (−2.770–−1.279)	< 0.001	67.18	1.5 ± 0.16	3.0 ± 0.31	< 0.001	**62.9**
T. arvense	8	26.566 (-)	0.000 (-)	1	4	inf	inf	1	4
T. inodorum	8	2.357 (1.528–3.185)	−0.860 (−1.101–−0.619)	< 0.001	141.61	2.7 ± 0.25	6.2 ± 0.52	< 0.001	**127.15**
U. dioica	8	2.777 (1.835–3.719)	−0.265 (−0.432–−0.097)	0.001	**158.69**	10.5 ± 1.91	21.6 ± 5.40	0.015	163.42
U. urens	6	2.284 (1.380–3.188)	−0.781 (−1.073–−0.489)	< 0.001	**130.70**	2.9 ± 0.28	6.7 ± 0.81	< 0.001	135.24

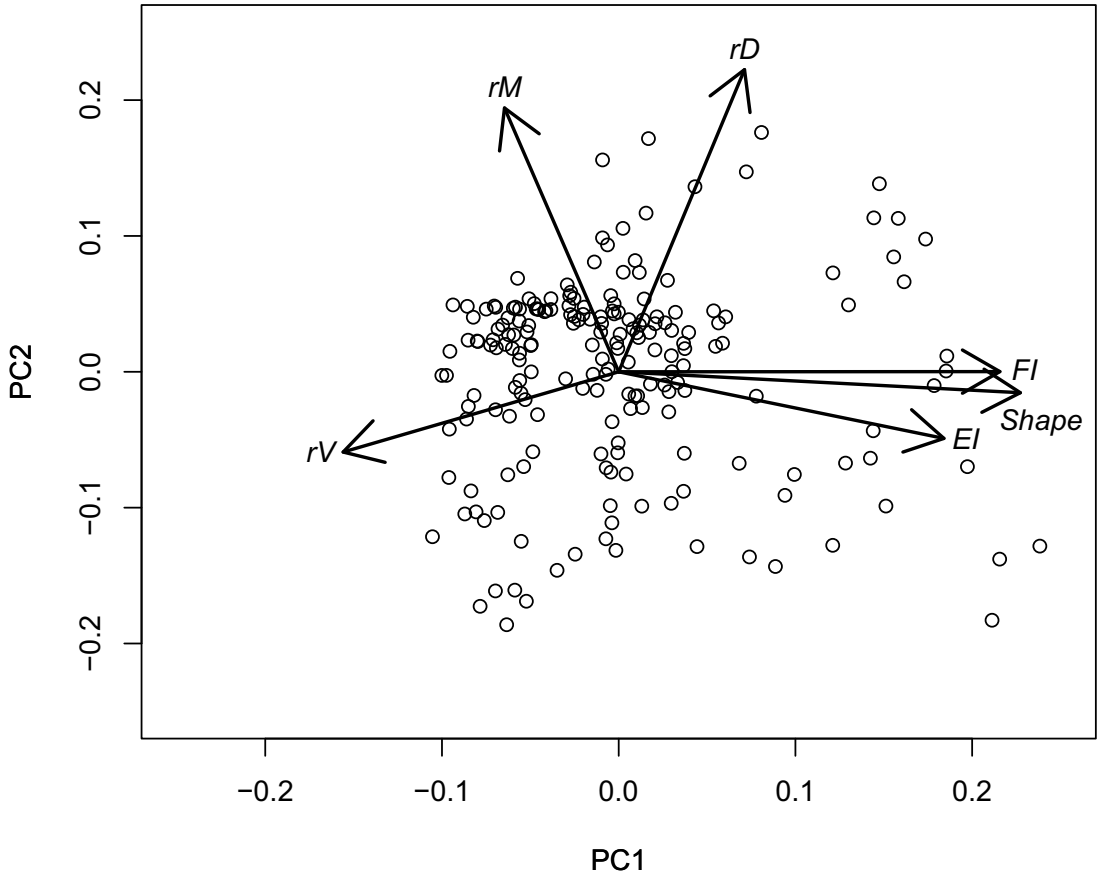

Figure 4. Principal component analysis in variation in seed form across species of seed and year after burial (*YAB*). PC1 and PC2 together explain 75.7% of the variance. Symbols indicate combination of seed species and *YAB*.

The persistence times of particular seed species in soil predicted based on the ICT are shown in Table 2. In some species, such as *A. sagittata*, the PT_{50} was predicted to be as low as 1 year, while in *G. urbanum*, the PT_{50} reached 10 years. The PT_{05} ranged from 2 (*C. glaucum*) to more than 20 years (*U. dioica* and *P. major*). As all seeds were found to be viable in *T. arvense*, the persistence in soil could not be estimated for this seed based on our data.

Across the species, predicted persistence was not related to *M*, *V*, *D* nor any of the three indexes for seed forms, using data for control seeds (results not shown). On the other hand, the PT_{50} was negatively related to the *rM* of buried seeds (Figure 5a). The relationship of the *rM* with PT_{50} for *YAB 1* and *YAB 2* cohorts was much steeper compared to the remaining *YAB* cohorts, suggesting that the seed species losing their mass in the first two years of burial survive in soil relatively shorter. The results were also similar for PT_{05}, with the exception of the insignificant relationship with *YAB 1* (not shown). The relative volume of neither cohort significantly explained the PT_{50} nor PT_{05} (not shown), and neither did the relative density of *YAB 1* and *YAB 2*. In contrast, seed persistence was negatively affected by the decrease in relative density in cohorts of *YAB 3–8* (Figure 5b), suggesting that seeds that lose relatively more mass than volume suffer from shorter lifespan in soil. Change in seed form indexes seemed to affect the predicted persistence (Figure 5c), but the effect was due to the outlying *G. urbanum* seed, which greatly changed its shape as a result losing seed coat and its appendages, so the trends were no longer significant when this species was removed from the analyses.

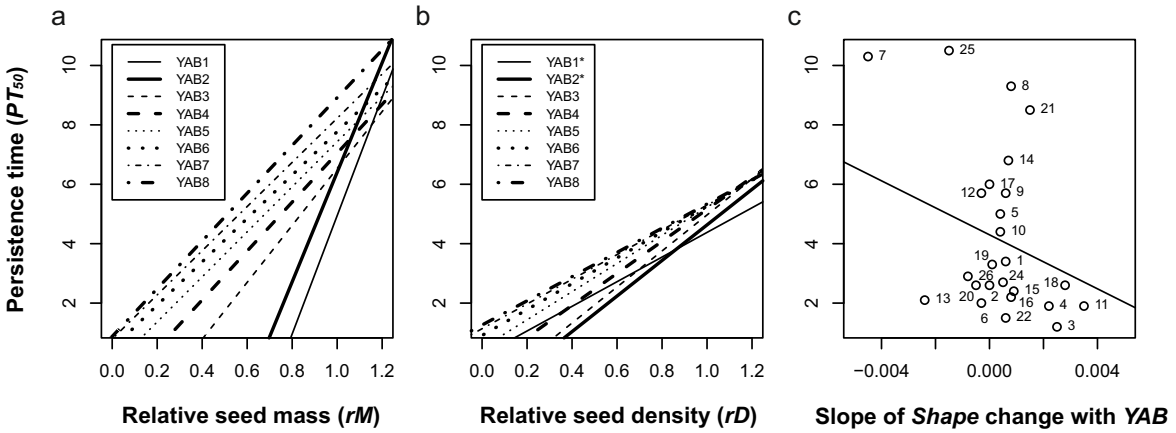

Figure 5. Effects of changes in selected morphological seed traits in particular cohorts on PT_{50}. (**a**) Relative seed mass (only regression slopes are shown); (**b**) Relative seed density (only regression slopes are shown; * slopes not significantly different from 0); (**c**) Slope of change in seed shape over years after burial (*YAB*). Symbols indicate seed species, for species codes, see Table 1.

4. Discussion

The seed characteristics investigated in this study involve traits that directly affect the fitness of plant species as well as traits that are most likely relevant only with respect to the perception and attractiveness of seeds to seed predators. The seeds were generally losing viability gradually over time when buried in the soil, and this loss of viability was accompanied by more or less noticeable morphological changes. Of these changes, the loss of mass and volume were the most prominent, although changes in other traits (seed density and shape) appeared to be significant in some species of seeds as well.

Seed persistence. Seed viability is known to non-linearly decline with time spent in soil as seeds decay or age [14,17,25,42], and this was true for the vast majority of species included in this study. Exponential decay rates were previously used for fitting decline in seed viability and for estimating the annual decay rate [14,43]. However, the visual inspection of the plots in the abovementioned studies clearly shows that the exponential curve was not capable of catching the uneven decline rate over time (see, e.g., Figure 1 in Conn et al. [43], plots for *C. bursa-pastoris* or *T. inodorum*, among others), resulting in the prediction of unrealistic annual decay rates. In this paper, we took advantage of using GLM with a binomial distribution of errors, which is a linearized form of a logistic regression, for describing the course of decline in seed viability, approximated by using the change in the proportion of persistent seeds in artificial seed banks over time. The method for analysing and predicting the persistence of seeds from soil seed banks at the population level was previously used, e.g., in work of Pakeman et al. [17], and we encourage others to do so as well.

We found this method to be robust, well grounded in mathematical theory, and easy to perform and interpret, and more importantly, it can be used for modelling seed persistence in soil. The greatest advantage of this approach is that one can describe the dynamics of seed persistence in time (or with any other variable that may have influenced the persistence, such as mass also used in this study) in a realistic way and estimate the persistence times. Using this method more frequently, the estimation of seed persistence in soil would approximate the reality closer, and would be more comparable among studies. The prerequisite for using this method is that a proportion of a seed population at each time point must be known, as estimated by a subset of the retrieved seeds, as in this study. Another advantage is that by using the appropriate distribution and link function, no data transformation is needed. Other researchers used various logistic regression models for analysing the decay/survival of seeds in soil [42,44] or complicated probit-based models [25]. Depending on the nature of their data (either Poisson or binomial distribution of errors), various data transformations were applied to approach counts (Poisson) or proportions (binomial) to Gaussian distribution, allowing for fitting the

chosen model. Although fitting some of these models may provide seed persistence curves and hence help with making predictions, we argue that GLM with binomial error structure shall be the preferred method in such studies due to its simplicity and accuracy, and this should be taken into consideration already when seed persistence data are collected.

We consider that wider application of logistic regression models in seed bank persistence studies would greatly increase our understanding of the dynamics of seed banks and that this approach represents a major step forward from classifying species into groups according to the detected persistence of their seeds, e.g., transient (< 1 year), short persistent (1–5 years) and long persistent (> 5 years) [2]. Such classification might erroneously suggest that seed persistence in soil is a discrete process, although the logistic approach has demonstrated that losing viability is continuous process and that a minor proportion of seed population may persist for a long time. For example, it can be predicted for *C. trachelium*, one of the least persistent species in our study, that 2.1 seeds out of a hypothetical 10^5 initial seeds will survive for five years in the soil, using the parameters describing the course of change in the proportion of persistent seeds estimated here, and their detection (and classification in the respective persistence class) depends on the sampling method, sample sorting precision and, of course, coincidence of finding viable seeds in soil samples. Therefore, literature data on seed persistence are extremely variable for each species (see, e.g., database of Thompson et al. [2]), and it is not rare that a species is classified as transient in one study and long persistent in another (e.g., *A. retroflexus* used also in this study according to the database of Thompson et al. [2]). There are obvious ecological reasons that may have caused such differences, including interpopulation or geographical variation, soil conditions and microflora [9,12,15,17,45], but such variation can also be largely attributed to the methods used for data collection (burial vs. emergence, soil depth) and evaluation. It is a well-established fact that seeds tend to germinate earlier and thus remain in a seed bank for less time when buried at shallow depths compared to deeper burial [25]. It also seems that seed emergence data are less reliable than seed burial experiments basically because species with larger seed production are more likely to be detected in soil for a longer period of time [46]. To address the variability among studies, several authors developed indexes [4,8,19] that consider the relative frequency of studies reporting a class of seed persistence and unite the known persistence data in one number. However, these indexes are inherently biased when different methods of seed persistence evaluation are combined for calculation [46] and therefore ecologically meaningless, despite their frequent use in ecological papers. For the sake of demonstration, we calculated the longevity index [4,19] and seed persistence index [8] for the 14 species for which data were available using only the burial data from the database of Thompson et al. [2]. The variation in the indexes values were in fact minor among species (Table 1), and all of them could be classified as long persistent if one would wish to do so, despite the variation in persistence times found in our study. In fact, papers that would provide reliably calculated predictions of seed persistence times are scarce, making difficult any comparison with persistence times PT_{50} and PT_{05} predicted in this study. For the species included in this study, we found only one reference [17], and interestingly, by using binomial GLM, the estimated PT_{50} for *P. lanceolata* (2.35 ± 0.43) was nearly identical to that from our study (2.2 ± 0.18). Others provided persistence times based on an exponential decay formula, but the resulting values are not comparable due to lack of fit (see above). We attempted to find patterns in the variation in persistence times and ecological explanation of these patterns by using morphological data of the control seeds as well as data describing the morphology change of seeds over time spent in soil. We found that predicted persistence times were unrelated to any of the morphological traits of the control seeds available, which contradicts the previously published literature data (e.g. [6,7,19,45,47]). Nevertheless, we found that a relative change in seed mass affected the predicted persistence. Seed species that lose mass rapidly already in the first two years of burial survive in soil relatively shorter. Seeds that lose relatively more mass than volume, i.e., seeds that have a rather strong seed coat, would suffer from a shorter life span in the soil as the embryo deteriorates. Additionally, relative seed mass explained the decline in the proportion of persistent seeds better than *YAB* in a significant portion of the species studied, mainly in those in which an increase in the proportion of persistent seeds occurred

after a period of decline. Such reversal periodically appears in the literature [14,21,22,26]) and is sometimes explained as a result of microsite variation in soil conditions favourable or disfavouring seed persistence. Additionally, the persistent times estimated in this study might be overestimating those that would occur in the field since these were based on identifiable seeds only and neglecting those which deteriorated completely. As seed mass generally declines with *YAB* non-linearly, using seed mass as an explanatory variable for the decline in the proportion of persistent seeds partly removed this scatter from the data. Congeneric species included in this study were expected to have similar persistence times because of common evolution and thus presumed taxonomic constraints on seed biology and similar life histories [12]. Contrary to this expectation, the variation among congeners was quite large in most cases; only the species of *Amaranthus* had rather similarly short persistence times. It is difficult to provide an explanation for such a scatter based on a limited number of species per genus (or family), and more likely, functional traits of plants and their habitat requirements might help to disentangle the ecological significance, if it exists, of such a striking scatter.

Seed morphology. The limited availability of descriptions of changes in external morphology and seed mass through time spent in soil is evident because these traits have not seemed to be important for species' ecology or economic importance. They may, however, have vital importance in determining the consumption rate of seed predators [48] and for identifying seeds from seed banks. We provide evidence that over the study period, the seed mass, volume and density largely become altered. There are several possible causes that result in changes in seed mass, volume, density and form through seed aging and decay. These relate to deterioration of the external structures, decay of the embryo and shrinking of the seed. As changes in specific descriptors of seed morphology were often correlated for a particular species of seed, the nature of the correlations provides insight into the manner of morphological changes the seeds underwent during soil burial. Significant change in seed shape indicators with *YAB* was caused by the reduction in volume. This can be a consequence of shrinking soft seeds due to death and deterioration of the embryo (e.g., *C. trachelium*), so the density remained unchanged. In a special case of *G. urbanum*, the seed coat of the control seeds is soft and equipped with a hook. Both structures deteriorate over time, which changes the overall seed shape and even increases seed density. Our results suggest that the seed coat does not have protective function in this species since *G. urbanum* was also one of the longest persistent species in our study. Many seeds did not change their volume over time in soil but mass (e.g., *A. powellii*), which indicates that these seeds are hard-coated with embryos dying and deteriorating. The fact that in some species of seeds the relative mass and volume was observed to "increase" after burial has to be viewed as a result of natural variability in seed size; alternatively it may partly be an artefact that the smallest seeds deteriorated first, so these were not available for taking morphological measurements.

The morphological changes and changes in seed mass over time spent in soil can also be well described by logistic regression in many species, and it seems plausible that this approach will find wide application in seed ecology studies. In addition to seed persistence [17,42,49] and morphology (this study), logistic models were recently applied for describing germination response to environmental conditions [50]. Thus, we propose using these models in situations in which the response of this type of seed to a focal variable can be expected.

5. Conclusions

In this study, we show on 26 species of seeds, how selected seed traits change with the course of burial in soil. Using this unique dataset, we described the patterns in the change of the proportion of persistent seeds and in morphology by using modern statistical methods such as logistic regression, which is a robust tool and provides comparable results across studies. Describing these changes using robust tools is important for obtaining better insight into seed bank biology and ecology and for making more accurate predictions of seed persistence of, e.g., arable weeds, and better plan their management.

Supplementary Materials: Appendix S1—Variability in seed traits over time spent in seed bank for 26 species of seeds. Supplementary materials can be found at http://www.mdpi.com/2073-4395/10/3/448/s1.

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