diversity

MDPI

Article

Soil Seed Bank of Alien and Native *Cornus* (Cornaceae) Taxa in Lithuania: What Determines Seed Density and Vertical Distribution in Soil?

Lukas Petrulaitis *, Valerijus Rašomavičius, Domas Uogintas and Zigmantas Gudžinskas

Institute of Botany, Nature Research Centre, Žaliųjų Ežerų Str. 49, 12200 Vilnius, Lithuania;
valerijus.rasomavicius@gamtc.lt (V.R.); domas.uogintas@gamtc.lt (D.U.); zigmantas.gudzinskas@gamtc.lt (Z.G.)
* Correspondence: lukas.petrulaitis@gamtc.lt

Abstract: Soil seed banks of alien plant species are sources of propagules that play a crucial role in plant population dynamics. Studies on seed banks of woody alien species are crucial for understanding mechanisms of their encroachment on natural habitats. This study aimed to compare vertical distribution, density and composition of seed banks formed by native *Cornus sanguinea* subsp. *sanguinea* and alien *C. alba*, *C. sericea* and *C. sanguinea* subsp. *australis* in the Southern Hemiboreal zone of Europe. Five sites for each of four taxa were selected for the study, and seeds were sampled using the soil core method (400 samples in two soil layers: the upper, 0–5 cm, and the lower, 5–10 cm). Extracted seeds were tested with tetrazolium chloride stain to assess their viability. Differences in the seed banks among taxa were compared using generalised linear mixed models (GLMM). The GLMM analysis revealed significant differences in soil seed bank densities in the upper soil between the studied taxa ($p < 0.001$). We found that two of the alien taxa (*C. alba* and *C. sanguinea* subsp. *australis*) formed a much denser seed bank containing more viable seeds than the native *Cornus sanguinea* subsp. *sanguinea*. All three alien species contained more viable seeds (from 40.7% to 45.2% in the upper soil layer) than the native *C. sanguinea* subsp. *sanguinea* (19.4% in the upper and 18.2% in the lower soil layer). The cover of *Cornus* and habitat type had no significant effect on the density of the seed bank, according to GLMM. This study supports the hypothesis that seed banks of alien *C. alba* and *C. sanguinea* subsp. *australis* are denser than those of native *C. sanguinea* subsp. *sanguinea*. Furthermore, the seed bank of alien taxa contained more viable seeds than the seed bank of *C. sanguinea* subsp. *sanguinea*. Results of this study contribute to the understanding of the invasiveness of alien *Cornus* taxa.

Keywords: alien taxa; habitats; intact seeds; seed density; viable seeds; woody species

check for updates

Citation: Petrulaitis, L.; Rašomavičius, V.; Uogintas, D.; Gudžinskas, Z. Soil Seed Bank of Alien and Native *Cornus* (Cornaceae) Taxa in Lithuania: What Determines Seed Density and Vertical Distribution in Soil? *Diversity* **2022**, *14*, 488. https://doi.org/10.3390/d14060488

Academic Editors: Michael Wink and Marcin W. Woch

Received: 11 May 2022
Accepted: 14 June 2022
Published: 15 June 2022

1. Introduction

Seed ecology, and specifically seed bank density and viability of seeds, are important determinants of plant competitive performance [1]. The soil seed bank in various habitats or certain plant species has been recognised as an essential element of plant ecology [2–4]. Soil seed banks are important in the conservation and management of endangered and invasive species and the restoration of habitats and plant communities [1,5,6].

Traditionally, a soil seed bank was classified as either transient, containing seeds that remained viable in the soil for less than a year, or persistent, including seeds that remained viable for more than a year [7]. However, the classification of soil seed banks has changed over the years, and the various types of soil seed banks have been defined [8]. The most widely accepted division of seed banks is into transient and persistent seed banks, which are further subdivided into two groups. A soil seed bank consisting of seeds that remain viable in the soil for more than one year, but less than five years, is recognised as a short-term persistent seed bank, whereas seed banks containing seeds that are viable for five or more years are recognised as long-term persistent seed banks [3,8]. The persistence of viable seeds in a soil seed bank depends on species' biological traits and environmental factors [9,10]. A high number of viable seeds in a seed bank can sustain the longevity and dynamics of a population, providing successful seedling emergence and recruitment [6].

Studies of the soil seed bank of an alien plant species can provide information on the invasiveness of a species in certain areas [11,12]. Masaki et al. [13] emphasised that the formation of persistent long-term seed banks could be advantageous for woody species in terms of colonising disturbed sites, especially for species whose recruitment depends on irregular habitat disturbances. The results of some studies suggest that the seeds of invasive plants tend to be better adjusted for survival in soil compared with native species [14]. Seed banks of invasive woody legumes (*Acacia*, *Cytisus*, *Genista*, etc.) have been studied extensively [1,15–17], whereas seed banks of fleshy-fruited woody species received much less attention [18–20]. Recent studies have revealed that alien woody plant species form denser soil seed banks in the invaded range than in their native ranges [21]

When seeds reach the soil and enter the seed bank, they gradually lose their germination potential, especially if they are short-lived [22]. Moreover, seeds may be affected by animals and microorganisms, which can influence their viability and survival in soil [23]. The proportions of damaged, intact, non-viable and viable seeds in soil seed banks show the relationship between seed input into the soil seed bank and its depletion [24,25]. On the other hand, the proportion of damaged, non-viable and viable seeds also gives some indication of the type of seed bank [26].

Most fleshy-fruited shrubs, including *Cornus* L., form transient seed banks [27]. However, studies on *C. controversa* Hemsl. suggest different patterns of seed persistence, as they remain viable in the soil for up to ten years [13]. Although the seed bank of *C. sanguinea* has been classified as transient since the seeds were found to be viable for less than a year [3], recent studies have revealed that seeds of this species can remain viable for at least three years under certain storage conditions [28]. Thus, these results suggest that seed banks of *C. sanguinea* could be treated as short-term persistent. Information on the seed banks of other species of the genus *Cornus*, including the widespread and invasive *C. alba* L. and *C. sericea* L., is almost unavailable [29,30]. In contrast, information on the seed bank of *C. sanguinea* L. is fragmentary and is typically presented as a secondary output of ecological studies of the overall seed bank in specific habitats [27]. A very dense seed bank of *C. sanguinea* containing intact seeds was found in Germany; however, the proportion of viable seeds extracted from the soil samples was less than 1% of the total number of intact seeds [5]. Experiments with *C. sanguinea* from Central and South Europe have given different rates of fresh seed germination, ranging from 53.0% to 90.8% [27,31,32].

As a part of the study of the ecological performance of alien and native *Cornus* taxa in the Southern Hemiboreal zone of Europe, we aimed to analyse the soil seed bank of four *Cornus* taxa occurring in Lithuania (*C. alba* L., *C. sericea* L., *C. sanguinea* L. subsp. *australis* (C.A. Mey.) Jáv. and *C. sanguinea* L. subsp. *sanguinea*). In the study area, *Cornus sanguinea* subsp. *sanguinea* is native, whereas the other taxa are naturalised aliens [30,33,34]. Studies on the soil seed bank of woody species confirmed that they form denser seed banks in the alien range because of more significant seed production and lower seed predation than in the native range [21]. Therefore, we set out to test the hypothesis that alien *Cornus* taxa also form a denser seed bank than the native representatives of this genus. This study aimed to compare vertical distribution, density and composition of the seed bank formed by native and alien *Cornus* in the Southern Hemiboreal zone of Europe. In this study, we addressed the following questions: (a) What is the vertical distribution of seeds in the soil layers? (b) What is the seed density in the soil seed bank, and how does it differ between native and alien *Cornus* taxa? (c) What is the viability of seeds extracted from the soil seed bank?

2. Materials and Methods

2.1. Studied Taxa

Most species in the genus *Cornus* are well defined and can be easily recognised; however, the taxonomy of several groups of species is still a subject of discussion. The taxonomy of *C. alba* and *C. sericea* for a long time has been controversial. In some periods, they have been treated as a single species [35]; *C. sericea* has been merged with *C. alba* at the rank of subspecies [36]. However, recent studies have suggested that they are phylogenetically divergent sister species [37,38]. In this study, we accept the later view and treat *C. alba* and *C. sericea* as separate species. *Cornus sericea* is a potentially invasive species that penetrates into natural forests, wetlands and other habitats across Europe [29].

For the soil seed bank studies, we carefully selected populations of unambiguously identified *C. alba*, *C. sericea*, *Cornus sanguinea* subsp. *sanguinea* and *C. sanguinea* subsp. *australis*. Plants were determined according to their morphological characteristics. *Cornus alba* and *C. sericea* we distinguished by the shape of seeds. *Cornus alba* has stones ellipsoid and tapered to a flat base, whereas *C. sericea* stones are subglobose and rounded at the base [37,39]. Subspecies of the *C. sanguinea* are easily distinguished by the leaf indumentum. The lower leaf surface of *C. sanguinea* subsp. *sanguinea* is covered with simple erect hairs, whereas leaves of *C. sanguinea* subsp. *australis* are covered with strongly appressed medifixed hairs [33,40].

The studied taxa of the genus *Cornus* are shrubs or small trees. *Cornus alba* is native to the continental regions of temperate and boreal Eurasia and occurs in mesic and wet meadows, on forest edges and shrubby thickets along rivers, and often invades various anthropogenic habitats [33,34]. *Cornus sericea* originates from North America; in its natural range, it occurs from Alaska to California. It usually occupies relatively moist habitats such as wet meadows, riparian zones, lakeshores, wetlands and swamp alder woodlands, both in native and alien ranges [37,41]. *Cornus sanguinea* subsp. *sanguinea* is widely distributed over the temperate regions of Europe, whereas *C. sanguinea* subsp. *australis* is native to south-eastern Europe and West Asia [33,40].

Cornus sanguinea subsp. *sanguinea* and *C. sanguinea* subsp. *australis* grow in broadleaved woodlands, along forest margins, and on the slopes of hills and ravines with calcareous soil [27]. However, in the non-native range, *C. sanguinea* subsp. *australis* occurs in semi-natural or even anthropogenic habitats, where it later penetrates into natural habitats [30].

2.2. Study Sites

Populations of the *Cornus* taxa were selected throughout Lithuania depending on their distribution (Figure 1, Table 1). Five populations of each taxon (*C. alba*, *C. sanguinea* subsp. *australis*, *C. sanguinea* subsp. *sanguinea* and *C. sericea*) were selected, giving a total of 20 sites. Stands with a substantial presence of the mature *Cornus* individuals in the shrub layer (with coverage ranging from 30% to 60%) and occupying at least 50 m^2 were selected for the seed bank sampling.

Voucher specimens from the studied populations were collected and deposited at the Herbarium of the Institute of Botany of the Nature Research Centre (BILAS) in Vilnius. As a result, habitat types were identified (applying the third level) and named following the EUNIS Habitat Classification [42] (Table 1).

Most study sites were selected from broadleaved deciduous forest (T1) habitats (10 sites). Five stands occurred in tree-dominated human-made habitats (V6), three in temperate and Mediterranean montane scrub (S3) and two in riverine and fen scrub (S9). For seed bank analysis among habitat types, we grouped seed bank data according to habitat types by higher level, such as scrub (S), forest (T) and vegetated human-made (V) habitats.

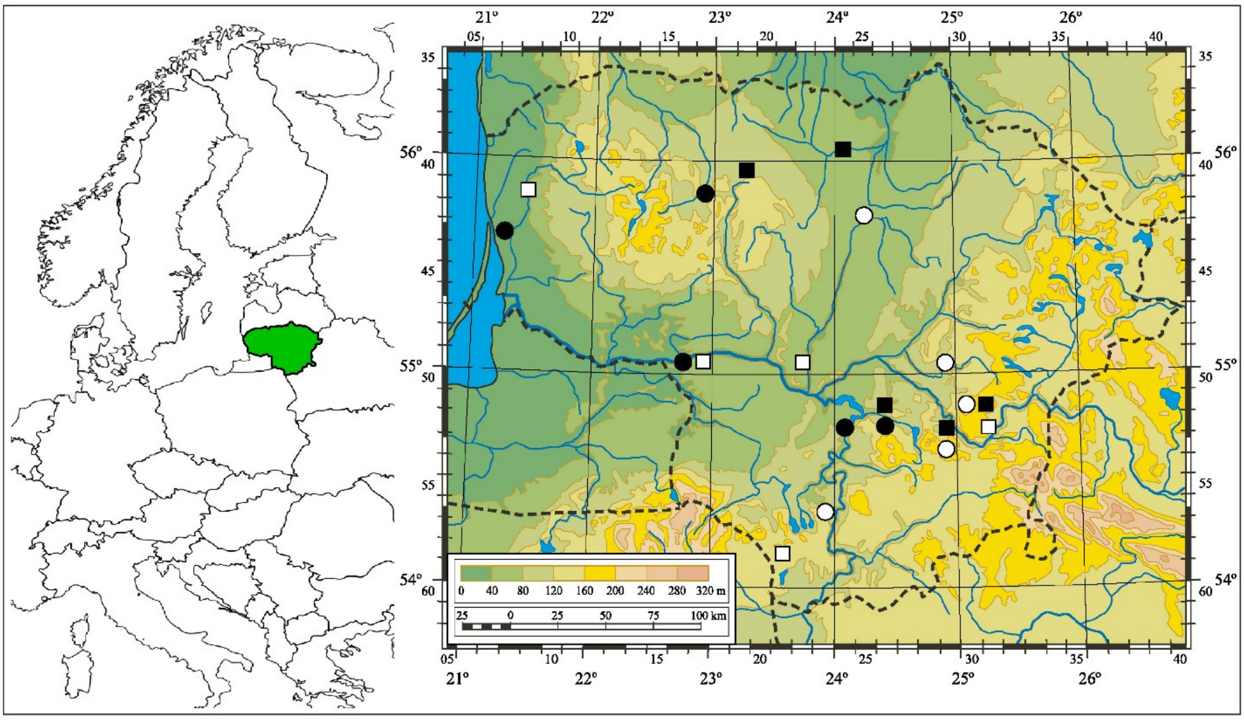


Figure 1. Location of the *Cornus* seed bank sampling sites in Lithuania. White dots—*C. alba*; black dots—*C. sericea*; black squares—*C. sanguinea* subsp. *australis*; white squares—*C. sanguinea* subsp. *sanguinea*.

Table 1. List of the study sites of the *Cornus* soil seed bank in Lithuania, with their administrative position, geographical coordinates and EUNIS habitat types.

Site Name	Administrative Unit	Longitude (°E)	Latitude (°N)	EUNIS Habitat Type
		Cornus alba		
Panevėžys	Panevėžys city	55.75270	24.31168	S92
Surgėliai	Širvintos distr.	55.01496	24.90851	S92
Kiemeliai	Vilnius distr.	54.85709	25.01377	T1J
Trakai	Trakai distr.	54.65630	24.90519	V64
Alytus	Alytus city	54.38806	24.00010	T1J
		Cornus sericea		
Juodlė	Kelmė distr.	55.81489	22.92023	T1F
Klaipėda	Klaipėda city	55.65867	21.23459	T12
Jurbarkas	Jurbarkas city	55.08171	22.72513	V64
Žiežmariai	Kaišiadorys distr.	54.81564	24.44084	T1J
Darsūniškis	Kaišiadorys distr.	54.74778	24.13509	T12
		Cornus sanguinea subsp. *sanguinea*		
Raguviškiai	Kretinga distr.	55.85995	21.39046	T11
Šilinė	Jurbarkas distr.	55.08856	22.94780	T1B
Piepaliai	Kaunas distr.	55.07423	23.78337	S35
Vilnius	Vilnius city	54.75331	25.29198	T1E
Liūnelis	Lazdijai distr.	54.12713	23.66146	S35
		Cornus sanguinea subsp. *australis*		
Švobiškis	Pasvalys distr.	56.05463	24.16271	V64
Šiauliai	Šiauliai city	55.93154	23.28444	S35
Kaišiadorys	Kaišiadorys city	54.87099	24.43677	V64
Maišiagala	Vilnius distr.	54.86959	25.05138	T1J
Vievis	Elektrėnai distr.	54.77094	24.83838	V64

2.3. Sampling Procedures

Soil samples for the seed bank analysis at the *Cornus* sites we collected in late autumn, during October and November of 2019, following a recommendation by Csontos [43], when the fruits had fallen from the plants. Sampling plots were arranged in transects across the *Cornus* stand (the transect length was 12 m). The sampling plots in the transect were set with 0.5 m gaps, and 20 soil cores (hereafter referred to as samples) were collected using a steel frame with sides 10 × 10 cm and height 10 cm. We carefully removed organic matter (fallen leaves, withered grass, plant debris) from core sampling from each plot surface.

Each collected soil core was divided into two layers (hereafter referred to as subsamples): the upper 0–5 cm and the lower 5–10 cm layer. Collected soil cores were transferred to labelled bags (with an indication of the taxon name, locality and layer) and brought to the laboratory. In the laboratory, the soil samples were slightly shredded to prevent soil clumping and left to dry in opened bags for about three months at ambient temperature (ca. 20 °C).

2.4. Seed Bank Analysis

The dried soil subsamples were crushed by hand, and the seeds were separated using a set of soil sieves (mesh sizes 3, 2, 1 and 0.25 mm). The seeds of the *Cornus* from each subsample were picked with pincers and placed into separate labelled paper bags. These seeds were later analysed using a binocular microscope and divided into intact seeds (with no visual damage to the shell) and damaged seeds (with visible damage to the shell). Intact and damaged seeds from each subsample were counted. A total of 400 soil samples and 800 subsamples were analysed during this study.

2.5. Tests of Seed Viability

The viability of all intact seeds extracted from the soil seed bank was tested by applying a standard staining method (TTC-test) using a 1% solution of 2,3,5 triphenyl tetrazolium chloride in distilled water [4]. Before testing, seeds were scarified by cutting off the tip of each seed shell with a medicinal scalpel to expose the embryo to enable better and faster TTC solution penetration. Then, scarified seeds were placed into test tubes with TTC solution and kept in the dark at a temperature of 35 °C for 24 h. For an evaluation of the embryo colour, a cross-section of the seed was made. Embryos were considered viable if they had carmine staining; slightly pink or white embryos were supposed to be non-viable [44].

2.6. Statistical Analyses

For the analysis of the seed density, seed counts per subsample (0.01 m^{-2}) were re-calculated to obtain mean seed density for a square metre of the study site (seeds 1 m^{-2}). The seed bank data from five sites of each studied taxon were pooled to determine differences between taxa and soil layers.

The results of descriptive statistics include mean values and standard deviations (mean ± SD). The normality of the data distribution was evaluated using the Shapiro–Wilk test. Since all of the datasets for the seed banks were non-normally distributed, non-parametric tests were applied. The differences between the sample medians were assessed by applying the Kruskal–Wallis H-test, and Dunn's z test was used for a post hoc pairwise comparison. The significance level of the statistical tests was set at $p < 0.05$. Differences in the proportions of intact seeds in the upper and lower soil layers between different taxa were assessed applying the chi-square (χ^2) test. Tests and descriptive statistics were performed using PAST 4.06 software [45]. Comparing seed banks of different *Cornus* species, the generalised linear mixed model (GLMM) was applied. The number of seeds in a seed bank was included as a response variable, the taxon was considered as a fixed factor, while the site, the cover of *Cornus* and habitat type (at the first level of classification) were considered as random effects in the model. The models were built in R-project environment, version 4.1.1 [46], using glmmADMB package, version 0.8.0 [47].

3. Results

3.1. Vertical Seed Distribution

A total of 1183 seeds, including both intact and damaged seeds, were collected from soil samples at the *Cornus alba* study sites, 433 seeds at the *C. sericea* sites, 712 seeds at the *C. sanguinea* subsp. *sanguinea* sites and 1547 seeds at the *C. sanguinea* subsp. *australis* sites (Table 2). Most of the seeds were concentrated in the upper soil layer (0–5 cm), a trend observed for all taxa and at all study sites, however, with some quantitative differences between the studied taxa. At *C. alba* and *C. sericea* sites, 93.3% and 92.8%, respectively, of all seeds were found in the upper soil layer (Table 2), and no significant differences were revealed between these species (χ^2 = 0.01, p = 0.93). It should be noted that no seeds were found in the lower soil layer at the Klaipėda site of *C. sericea*. The proportions of seeds in the upper soil layer at *C. sanguinea* subsp. *sanguinea* and *C. sanguinea* subsp. *australis* sites were 88.6% and 87.1%, respectively, and no significant differences were found between taxa (χ^2 = 0.84, p = 0.36).

Table 2. Total number of seeds in both upper and lower soil layers, number and percentage of intact and damaged as well as viable and non-viable seeds of the studied *Cornus* taxa in the upper and lower soil layers.

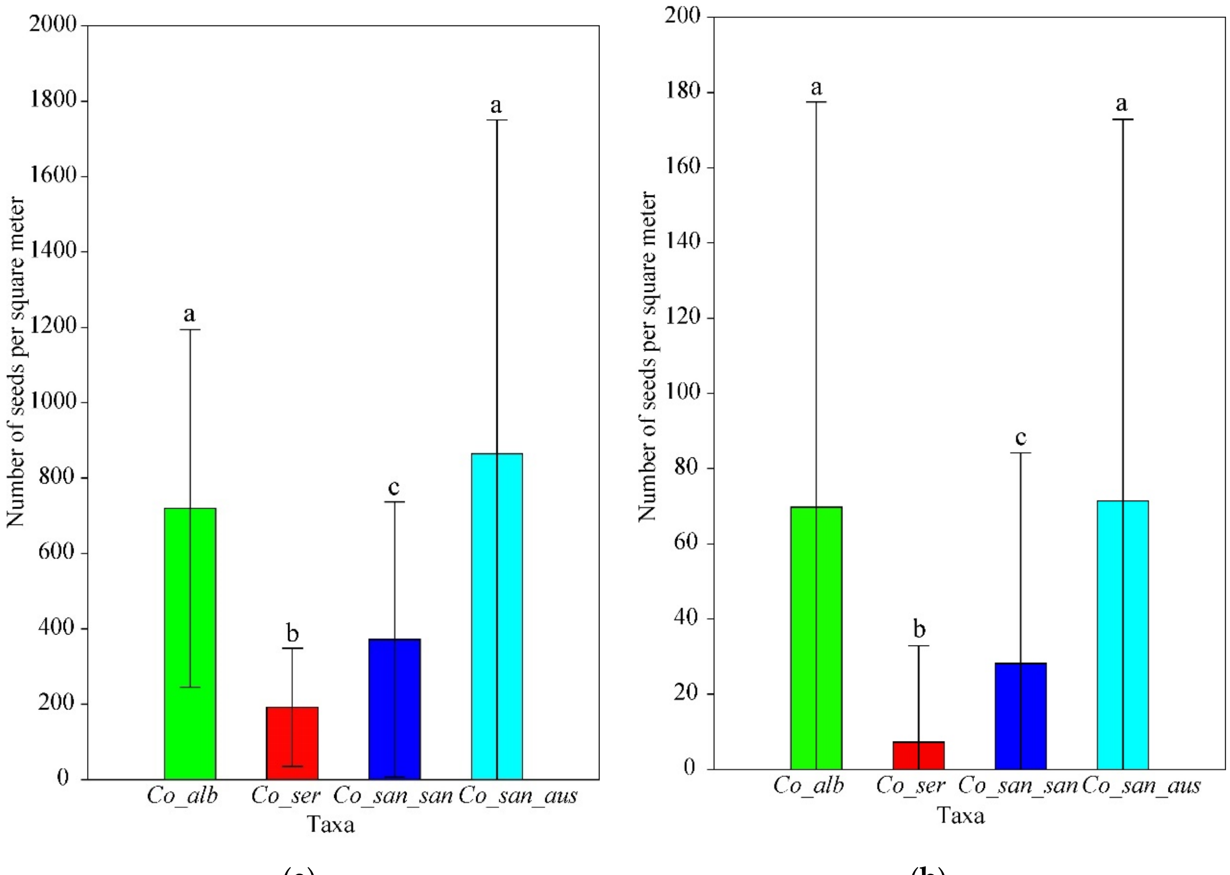
Taxa	*C. alba*	*C. sericea*	***C. sanguinea* subsp. *sanguinea***	***C. sanguinea* subsp. *australis***
Total number in both layers	1183	433	712	1547
Upper soil layer (0–5 cm)				
Total number of seeds	1100	402	631	1347
Percentage of the total number of seeds	93.3	92.8	88.6	87.1
Number of damaged seeds	318	168	201	317
Percentage of damaged seed in the layer	28.9	41.8	31.9	23.5
Number of intact seeds	782	234	430	1030
Percentage of intact seed in the layer	71.1	58.2	68.1	76.5
Number of viable seeds	318	96	80	466
Number of non-viable seeds	464	138	350	564
Lower soil layer (5–10 cm)				
Total number of seeds	83	31	81	200
Percentage of the total number of seeds	6.8	7.2	11.4	12.9
Number of intact seeds	74	10	44	117
Percentage of intact seed in the layer	89.2	38.7	54.3	58.5
Number of damaged seeds	9	19	37	83
Percentage of damaged seed in the layer	10.8	61.3	45.7	41.5
Number of viable seeds	39	6	8	52
Number of non-viable seeds	35	4	36	65

The highest percentage of damaged seeds (41.8%) in the upper soil layer was recorded at *C. sericea*, whereas at *C. sanguinea* subsp. *australis* damaged seeds comprised 23.5% of all seeds from this layer (Table 2). In the lower soil layer, the total number of seeds was significantly lower than in the upper soil layer, and a higher percentage of seeds were damaged than in the upper layer. The proportion of damaged seeds for all studied taxa was higher in the lower soil layer, except for *C. alba* (10.8%).

Most intact seeds were concentrated in the upper soil layer at all study sites (Table 2). However, significant differences were found between taxa concerning the absolute number of intact seeds. For example, at *C. alba* sites, 782 intact seeds were found in the upper soil layer, whereas at *C. sericea* sites, 234 intact seeds were found. At the sites of native *C. sanguinea* subsp. *sanguinea*, 430 intact seeds were extracted from the upper soil layer subsamples, whereas at *C. sanguinea* subsp. *australis* sites, 1030 intact seeds were found in this layer. In the lower layer, the number of intact seeds was low, but it was higher than the number of damaged seeds at the study sites of all taxa except *C. sericea* (Table 2).

3.2. Seed Bank Density

The highest mean density of seeds in the upper layer was found for *C. sanguinea* subsp. *australis*, whereas the lowest mean number of seeds was revealed at *C. sericea* sites (Figure 2). The same trend was recorded in the lower soil layer. The highest mean density of seeds in the lower soil layer occurred at *C. sanguinea* subsp. *australis* sites, whereas the lowest density was at *C. sericea* sites.



(**a**) (**b**)

Figure 2. Mean number of the intact *Cornus* seeds per square metre: (**a**)—upper layer (0–5 cm); (**b**)—lower layer (5–10 cm). Whiskers indicate standard deviation. Different letters above the whiskers indicate significant differences ($p < 0.05$) applying Dunn's z-test. Abbreviations of taxa: Co_alb—*Cornus alba*; Co_ser—*Cornus sericea*; Co_san_san—*Cornus sanguinea* subsp. *sanguinea*; Co_san_aus—*Cornus sanguinea* subsp. *australis*.

The number of intact seeds and their density in the upper soil layer (0–5 cm) varied greatly between the sites. The highest density of intact seeds in this layer was recorded at *C. alba* sites, whereas the lowest density of intact seeds was found at *C. sericea* sites

(Table 3). The density of intact seeds at all pooled *C. alba* sites was significantly higher than at *C. sericea* sites (Dunn's $z = 7.62$, $p < 0.001$). The largest variation in the mean density of intact seeds in the upper soil layer was recorded at *C. sanguinea* subsp. *australis* sites. The density of intact seeds at *Cornus sanguinea* subsp. *australis* sites was significantly higher than the density of intact seeds at *Cornus sanguinea* subsp. *sanguinea* sites ($z = 4.11$, $p < 0.001$). A significant difference in the intact seed density in the upper soil layer was found between all studied taxa by applying the Kruskal–Wallis H test (H = 75.35, $p < 0.001$). However, a pairwise comparison revealed no differences between *C. alba* and *C. sanguinea* subsp. *australis* sites ($z = 1.02$, $p = 0.306$).

Table 3. Mean density of intact, viable and non-viable seeds (mean ± SD) per 1 m^{-2} and percentage of viable and non-viable seeds in the upper soil layer (0–5 cm) of individual sites and all sites pooled of the studied *Cornus* taxa. Different lower-case and capital letters denote statistically significant differences between the means among the sites and taxa, respectively, applying Dunn's post hoc test.

Site Name	Intact Seeds	Viable Seeds	Non-Viable Seeds	Viable Seeds (%)	Non-Viable Seeds (%)
		Cornus alba			
Panevėžys	1095 ± 480 ^{a}	430 ± 267 ^{a}	665 ± 283 ^{a}	39.3	60.7
Surgėliai	555 ± 361 ^{b}	250 ± 191 ^{ab}	305 ± 199 ^{b}	45.0	55.0
Kiemeliai	590 ± 346 ^{b}	185 ± 224 ^{b}	405 ± 246 ^{b}	31.4	68.6
Trakai	415 ± 308 ^{b}	185 ± 182 ^{b}	230 ± 210 ^{b}	44.6	55.4
Alytus	1255 ± 857 ^{a}	540 ± 554 ^{a}	715 ± 434 ^{a}	43.0	57.0
All sites pooled	782 ± 601 ^{A}	318 ± 348 ^{A}	464 ± 348 ^{A}	40.7	59.3
		Cornus sericea			
Juodlė	240 ± 330 ^{a}	65 ± 79 ^{abc}	175 ± 268 ^{a}	27.1	72.9
Klaipėda	285 ± 323 ^{a}	180 ± 248 ^{ab}	105 ± 124 ^{a}	63.2	36.8
Jurbarkas	255 ± 161 ^{a}	30 ± 56 ^{ac}	225 ± 126 ^{b}	11.8	88.2
Žiežmariai	150 ± 105 ^{a}	80 ± 87 ^{abc}	70 ± 78 ^{a}	53.3	46.7
Darsūniškis	240 ± 266 ^{a}	125 ± 122 ^{b}	115 ± 174 ^{a}	52.1	47.9
All sites pooled	234 ± 252 ^{B}	96 ± 147 ^{B}	138 ± 177 ^{B}	41.0	59.0
		Cornus sanguinea subsp. *sanguinea*			
Raguviškiai	825 ± 795 ^{a}	215 ± 347 ^{a}	610 ± 500 ^{a}	26.1	73.9
Šilinė	120 ± 161 ^{b}	15 ± 36 ^{b}	105 ± 143 ^{b}	12.5	87.5
Piepaliai	615 ± 453 ^{a}	105 ± 156 ^{ac}	510 ± 356 ^{a}	17.1	82.9
Vilnius	70 ± 117 ^{b}	20 ± 51 ^{b}	50 ± 81 ^{b}	28.6	71.4
Liūnelis	525 ± 265 ^{a}	45 ± 59 ^{bc}	480 ± 238 ^{a}	8.6	91.4
All sites pooled	431 ± 517 ^{C}	80 ± 197 ^{B}	351 ± 386 ^{C}	18.6	81.4
		Cornus sanguinea subsp. *australis*			
Švobiškis	2280 ± 1248 ^{a}	585 ± 408 ^{a}	1695 ± 998 ^{a}	25.7	74.3
Šiauliai	345 ± 268 ^{bd}	170 ± 149 ^{b}	175 ± 176 ^{bd}	49.3	50.7
Kaišiadorys	605 ± 309 ^{c}	1390 ± 1810 ^{a}	345 ± 269 ^{ac}	80.1	19.9
Maišiagala	185 ± 193 ^{b}	130 ± 152 ^{bc}	55 ± 74 ^{d}	70.3	29.7
Vievis	1735 ± 1912 ^{cd}	55 ± 112 ^{c}	550 ± 291 ^{e}	9.1	90.9
All sites pooled	1030 ± 1315 ^{A}	466 ± 978 ^{A}	564 ± 769 ^{AC}	45.2	54.8

The results of GLMM rejected the presumption of the locality effect, but confirmed that the studied taxa have a significant effect on seed bank density (Table 4). However, the density of seeds in the lower soil layer was affected by the locality in the case of *Cornus alba* and *C. sanguinea* subsp. *australis*. The coverage of *Cornus* taxa has no significant effect on seed bank density in the upper soil layer, but depended on the taxon (Table 5). In the lower soil layer, the density of seeds of *Cornus alba* and *C. sanguinea* subsp. *australis* was affected by the coverage of *Cornus*, whereas the density of *C. sanguinea* subsp. *sanguinea* and *C. sericea* seeds did not depend on the cover of the individuals.

Table 4. GLMM results for the intact seed density in the upper (0–5 cm) and lower (5–10 cm) soil layers. Taxa were considered as a fixed effect (f), whereas locality was considered as a random effect (r). SE—standard error; SD—standard deviation.

Effects	Estimate	SE	*z*-Value	*p*-Value	Variance	SD
Upper layer						
f *C. alba*	1.966	0.298	6.60	<0.001		
f *C. sericea*	0.820	0.303	2.71	<0.01		
f *C. sanguinea* subsp. *sanguinea*	1.079	0.304	3.55	<0.001		
f *C. sanguinea* subsp. *australis*	1.839	0.299	6.15	<0.001		
r Locality					0.436	0.660
Lower layer						
f *C. alba*	−0.440	0.458	−0.96	0.336		
f *C. sericea*	−2.365	0.546	−4.33	<0.001		
f *C. sanguinea* subsp. *sanguinea*	−1.495	0.515	−2.90	<0.001		
f *C. sanguinea* subsp. *australis*	−0.414	0.468	−0.89	0.376		
r Locality					0.961	0.980

Table 5. GLMM results for the intact seed density in the upper (0–5 cm) and lower (5–10 cm) soil layers. Taxa were considered as a fixed effect (f) and the cover of *Cornus* was considered as a random effect (r). SE—standard error; SD—standard deviation.

Effects	Estimate	SE	*z*-Value	*p*-Value	Variance	SD
Upper layer						
f *C. alba*	1.939	0.241	8.04	<0.001		
f *C. sericea*	0.650	0.252	2.59	<0.01		
f *C. sanguinea* subsp. *sanguinea*	1.224	0.243	5.04	<0.001		
f *C. sanguinea* subsp. *australis*	1.988	0.242	8.22	<0.001		
r *Cornus* cover					0.279	0.528
Lower layer						
f *C. alba*	−0.399	0.498	−0.80	0.423		
f *C. sericea*	−1.167	0.573	−2.82	<0.01		
f *C. sanguinea* subsp. *sanguinea*	−1.313	0.519	−2.53	0.011		
f *C. sanguinea* subsp. *australis*	0.661	0.504	1.31	0.190		
r *Cornus* cover					1.127	1.062

The number of intact seeds in the lower soil layer (5–10 cm) was less variable (Table 6) than in the upper soil layer (Table 3). The lowest variation in the density of intact seeds was observed between *C. sericea* sites (Table 6). The density of intact seeds at pooled sites of *C. alba* was significantly higher than the density at *C. sericea* sites ($z = 4.69$, $p < 0.001$). The highest variation in the mean density of intact seeds in the lower soil layer was observed for the *C. sanguinea* subsp. *australis* sites. The density of intact seeds in the lower soil layer at *C. sanguinea* subsp. *sanguinea* sites was significantly lower than at *C. sanguinea* subsp.

australis sites (z = 3.08, p = 0.002). Significant differences in the density of intact seeds in the lower soil layer between all studied sites of pooled taxa were found by applying the Kruskal–Wallis H test (H = 23.61, $p < 0.001$). However, a pairwise comparison revealed no differences between *C. alba* and *C. sanguinea* subsp. *australis* sites (z = 0.86, p = 0.390).

Table 6. Mean density of intact, viable and non-viable seeds (mean ± SD) per 1 m^{-2} and percentage of viable and non-viable seeds in the lower soil layer (5–10 cm) of individual sites and all sites pooled of the studied *Cornus* taxa. Different lower-case and capital letters denote statistically significant differences between the means among the sites and taxa, respectively, applying Dunn's post hoc test.

Site Name	Intact Seeds	Viable Seeds	Non-Viable Seeds	Viable Seeds (%)	Non-Viable Seeds (%)
			Cornus alba		
Panevėžys	25 ± 64 ^{a}	10 ± 31 ^{a}	15 ± 49 ^{a}	40.0	60.0
Surgėliai	50 ± 100 ^{ab}	25 ± 64 ^{a}	25 ± 44 ^{ab}	50.0	50.0
Kiemeliai	90 ± 129 ^{a}	40 ± 82 ^{a}	50 ± 76 ^{ab}	44.4	55.6
Trakai	110 ± 137 ^{b}	60 ± 109 ^{a}	50 ± 61 ^{b}	54.5	45.5
Alytus	95 ± 123 ^{b}	60 ± 109 ^{a}	35 ± 49 ^{ab}	63.2	36.8
All sites pooled	74 ± 116 ^{A}	39 ± 84 ^{A}	35 ± 56 ^{A}	52.7	47.3
			Cornus sericea		
Juodlė	10 ± 31 ^{a}	10 ± 31 ^{a}	0 ^{a}	100	0
Klaipėda	0 ^{a}	0 ^{a}	0 ^{a}	0	0
Jurbarkas	15 ± 67 ^{a}	5 ± 22 ^{a}	10 ± 44 ^{a}	33.3	66.7
Žiežmariai	10 ± 30 ^{a}	10 ± 30 ^{a}	0 ^{a}	100	0
Darsūniškis	15 ± 37 ^{a}	5 ± 22 ^{a}	10 ± 31 ^{a}	33.3	66.7
All sites pooled	10 ± 43 ^{B}	6 ± 24 ^{B}	4 ± 24 ^{B}	60.0	40.0
			Cornus sanguinea subsp. *sanguinea*		
Raguviškiai	125 ± 141 ^{a}	30 ± 57 ^{a}	95 ± 94 ^{a}	24.0	76.0
Šilinė	0^{b}	0 ^{b}	0 ^{b}	0	0
Piepaliai	15 ± 37 ^{b}	5 ± 22 ^{b}	10 ± 31 ^{b}	33.3	66.7
Vilnius	5 ± 22 ^{b}	0 ^{b}	5 ± 22 ^{b}	0	100
Liūnelis	75 ± 107 ^{a}	5 ± 22 ^{b}	70 ± 103 ^{a}	6.7	93.3
All sites pooled	44 ± 94 ^{C}	8 ± 31 ^{B}	36 ± 75 ^{A}	18.2	81.8
			Cornus sanguinea subsp. *australis*		
Švobiškis	315 ± 287 ^{a}	95 ± 94 ^{a}	220 ± 238 ^{a}	30.2	69.8
Šiauliai	35 ± 59 ^{b}	35 ± 59 ^{bc}	0 ^{b}	100	0
Kaišiadorys	170 ± 210 ^{c}	110 ± 174 ^{ab}	60 ± 88 ^{c}	64.7	35.3
Maišiagala	5 ± 22 ^{bd}	5 ± 22 ^{c}	0^{b}	100	0
Vievis	60 ± 114 ^{a}	15 ± 37 ^{c}	45 ± 115 ^{bc}	25.0	75.0
All sites pooled	117 ± 201 ^{A}	52 ± 102 ^{A}	65 ± 147 ^{A}	44.4	55.6

The results of GLMM revealed no significant effect of the habitat type on the seed bank density; however, as in other cases, it reliably depends on the studied taxa (Table 7). Most of intact seeds were detected in vegetated human-made habitats (V), whereas in forests and other wooded land (T), numbers of intact seeds were lower (Table 8).

Table 7. GLMM results for the intact seed density in the upper (0–5 cm) soil layer. Taxa were considered as a fixed effect (f) and the habitat type was considered as a random effect (r). SE—standard error; SD—standard deviation.

Effects	**Estimate**	**SE**	***z*-Value**	***p*-Value**	**Variance**	**SD**
Upper layer						
f *C. alba*	2.014	0.146	13.81	<0.001		
f *C. sericea*	0.990	0.155	6.38	<0.001		
f *C. sanguinea* subsp. *sanguinea*	1.545	0.150	10.33	<0.001		
f *C. sanguinea* subsp. *australis*	1.971	0.146	13.53	<0.001		
r Habitat type					0.0579	0.2407

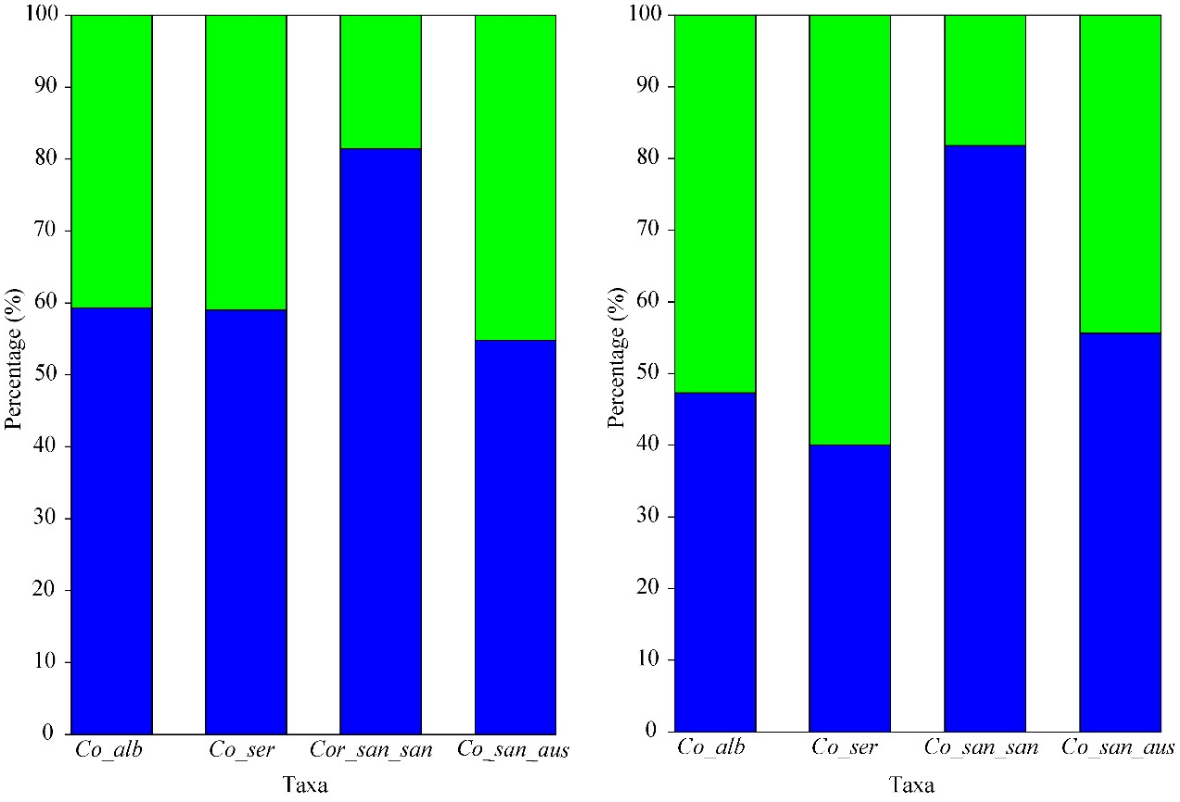
Table 8. Number of intact seeds of *Cornus* taxa in different habitats in the upper (0–5 cm) and lower (5–10 cm) soil layer. Different letters indicate significant differences ($p < 0.05$) applying Dunn's post hoc z-test. S—Heathlands, scrub and tundra; T—Forests and other wooded land; V—Vegetated human-made habitats.

Taxa	**Scrub (S)**	**Forest (T)**	**Human-Made (V)**
Upper layer			
C. alba	825 ± 500 ^{a}	923 ± 728 ^{a}	415 ± 308 ^{b}
C. sericea	–	229 ± 271 ^{a}	255 ± 161 ^{a}
C. sanguinea subsp. *sanguinea*	570 ± 369 ^{a}	337 ± 193 ^{b}	–
C. sanguinea subsp. *australis*	345 ± 268 ^{a}	185 ± 193 ^{a}	1540 ± 1485 ^{b}
Lower layer			
C. alba	38 ± 84 ^{a}	93 ± 125 ^{b}	110 ± 137 ^{b}
C. sericea	–	10 ± 34 ^{a}	20 ± 70 ^{a}
C. sanguinea subsp. *sanguinea*	45 ± 85 ^{a}	43 ± 100 ^{a}	–
C. sanguinea subsp. *australis*	35 ± 59 ^{a}	5 ± 22 ^{a}	182 ± 237 ^{b}

Analysis of the habitat type effect on the number of intact seeds in the soil seed bank of individual taxa revealed different pattern of relationships. We found that the upper soil layer of *C. sanguinea* subsp. *sanguinea* occurring in temperate scrub habitats contains a significantly higher number of seeds than in broadleaved deciduous forest habitats (Dunn's $z = 4.30$, $p < 0.001$). Different tendencies were observed at *C. sanguinea* subsp. *australis* sites. No significant differences were found between intact seed number in broadleaved deciduous forest and temperate scrub habitats ($z = 1.10$, $p = 0.27$), whereas significant differences in seed number were found between broadleaved deciduous forest and human-made habitats ($z = 5.85$, $p < 0.001$), as well as between temperate scrub and human-made ($z = 4.50$, $p < 0.001$) habitats. Thus, the number of intact seeds was higher in open or semi-open habitats than in woodland habitats. It was revealed that the pattern of intact seed density in the lower soil layer is the same as that in the upper soil layer (Table 8).

3.3. Seed Viability

After analysing the intact *Cornus* seeds, we found that viable seeds in the upper soil layer comprised between 8.6% and 80.1% of all intact seeds extracted from this layer at individual sites (Table 3). At *C. alba* sites, the density of viable seeds in the upper soil layer was lower than the density of non-viable seeds (Figure 3). The density of viable seeds in the seed bank of *C. sericea* sites was significantly lower than at the sites of other studied taxa. The proportion of viable seeds at the study sites of *C. sericea* ranged from 27.1% to 63.2% of the total number of intact seeds at the individual sites for this species.



(**a**) (**b**)

Figure 3. Ratio of viable (green) and non-viable (blue) seeds in different soil layers. (**a**)—in the upper layer; (**b**)—in the lower layer. Abbreviations of taxa: Co_alb—*Cornus alba*; Co_ser—*Cornus sericea*; Co_san_san—*Cornus sanguinea* subsp. *sanguinea*; Co_san_aus—*Cornus sanguinea* subsp. *australis*.

At the sites of the native *C. sanguinea* subsp. *sanguinea*, viable seeds comprised from 8.6% to 28.6% of all intact seeds (Table 3). In contrast, at the sites of the alien *C. sanguinea* subsp. *australis*, the soil seed bank was significantly denser ($z = 5.55$, $p < 0.001$) and contained a higher proportion of viable seeds than at the sites of *C. sanguinea* subsp. *sanguinea* ($\chi^2 = 16.97$, $p < 0.001$). Viable seeds in the upper soil layer at the sites of *C. sanguinea* subsp. *australis* comprised from 9.1% to 80.1% of all intact seeds. It should be noted that *C. sanguinea* subsp. *australis* and *C. alba* were not significantly different from each other ($z = 1.08$, $p = 0.280$) in terms of the density of viable seeds in the upper soil layer.

The number of seeds in the lower soil layer (5–10 cm) was much lower than in the upper layer (0–5 cm). Nevertheless, the lower soil layer contained viable seeds at many study sites (Table 6). At *C. alba* sites, viable seeds comprised from 40.0% to 63.2% of all intact seeds recorded in this layer. At *C. sericea* sites, the density of viable seeds in the lower soil layer was significantly lower than at *C. alba* sites ($z = 3.47$, $p < 0.001$). At two sites of *C. sericea*, Juodlė and Žiežmariai, all intact seeds in the lower soil layer were viable, although their numbers were small.

At the sites of the native *C. sanguinea* subsp. *sanguinea*, the density of viable seeds in the lower soil layer was relatively low, and they comprised from 0% to 33.3% of all intact seeds (Table 6). Contrasting results were obtained from the analysis of the soil seed bank in the lower soil layer at the sites of alien *C. sanguinea* subsp. *australis*, where viable seeds comprised from 25.0% to 100% of all recorded intact seeds. The density of viable seeds in the lower soil layer was significantly different between *C. sanguinea* subsp. *sanguinea* and *C. sanguinea* subsp. *australis* ($z = 4.62$, $p < 0.001$); however, no significant differences were found between the density of viable seeds at *C. sanguinea* subsp. *sanguinea* and *C. sericea* (z = 0.20, p = 0.838) sites.

4. Discussion

4.1. Vertical Seed Distribution

Analysis of the vertical distribution of seeds of the four studied *Cornus* taxa in the soil showed that seeds were concentrated in the upper soil layer, with a much lower number of seeds found in the lower layer. The high concentration of seeds in the upper soil layer is characteristic of many plant species [2,21,24]. The biological characteristics of the species determine the vertical distribution of seeds in the soil, primarily seed production, seed shape and size [10,48,49]. In addition, environmental factors such as the mechanical composition of the soil [48], the thickness and nature of the plant debris layer [22], the type of habitat [7] and the activity of small vertebrates, invertebrates and micro-organisms [22,50] also have a significant influence on the distribution of seeds in the soil.

We found that the proportion of seeds in the lower soil layer was higher for *C. sanguinea* subsp. *sanguinea* and *C. sanguinea* subsp. *australis* than *C. alba* and *C. sericea* (Table 2). We assume that the different proportions of seeds in the soil layers were caused by the different shapes of the seeds of the studied *Cornus* taxa. The influence of seed shape on the depth of penetration into the soil has been confirmed by analysing the seeds of different plant species. It has been confirmed that round seeds of shrub *Cytisus multiflorus* (L'Hér.) Sweet can penetrate the deeper soil layers more easily since this shape reduces the friction [48]. The seeds of both subspecies of *Cornus sanguinea* are globular or subglobular, whereas the seeds of *C. alba* and *C. sericea* are laterally compressed [51].

At Šilinė and Vilnius sites, the seed banks of *C. sanguinea* subsp. *sanguinea* were studied within broadleaved deciduous forest habitats. We found exceedingly small numbers of seeds in the upper soil layer, and intact seeds were absent from the lower soil layer. The thick layer of dead leaves and other plant remnants accumulating in broadleaved forests probably hampered seed penetration to the deeper soil layers. Some authors have also found that the surrounding vegetation influences seed penetration into the soil [22]. The absence of seeds in the lower soil layer at the Klaipėda site of *C. sericea* was also related to the peculiarities of the habitat. The permanently damp soil of the *Alnus glutinosa* (L.) Gaertn. stand could, in our opinion, have interfered with the activities of small soil vertebrates and invertebrates, which have a significant influence on seed migration in the soil [22,49,50]. We observed rodent activity at other study sites of *Cornus alba* and *C. sericea* with mesic soils.

4.2. Density of the Seed Bank

The study results showed a dense seed bank in the soil at the sites of the studied four *Cornus* taxa. The density varied between sites of the same taxon as well as between taxa. The highest density of intact seeds in both soil layers was recorded for *C. sanguinea* subsp. *australis*, whereas the lowest density was in the soil of *C. sericea* sites. It is known that a high yield of fruits per individual positively correlates with the seed density in the seed bank [52]. *Cornus alba* and *C. sericea* usually flower and yield twice during the growing season [37,53] and can, therefore, be expected to contribute more to the seed bank than *C. sanguinea*, which tends to flower and yield fruit once a year [27]. *Cornus alba* and *C. sericea* also flowered twice during the growing season at the study sites in Lithuania, although the fruit set after the second flowering of *C. sericea* was poor. This may explain why the seed bank density of the *C. sericea* was significantly lower than that of *C. alba*. It has been found that frugivore birds are a factor affecting the density of the seed bank through the removal of fruits [54]. Studies on *C. amomum* Mill. and *C. racemosa* Lam. in the native range in North America have shown that their fruits are consumed faster than fruits of non-native *Rhamnus cathartica* L. and *Rosa multiflora* Thunb [55]. Fruits of *Cornus amomum* and *C. racemosa* share similar morphological traits (shape and colour) with *C. alba* and *C. sericea*. We presume that their seed bank density is also affected by the activity of frugivore birds.

By comparing the density of intact seeds in the seed bank of the native *C. sanguinea* subsp. *sanguinea* and of the alien *C. sanguinea* subsp. *australis*, we found that alien subspecies formed significantly denser seed banks than the native subspecies in both analysed soil layers. Nevertheless, at the Piepaliai and Liūnelis sites of *C. sanguinea* subsp. *sanguinea*, the density of intact seeds in the upper soil layer was the same or even higher than at the Šiauliai and Maišiagala sites of *C. sanguinea* subsp. *australis*. Habitat characteristics can probably explain these differences in seed density in the soil. At the Piepaliai and Liūnelis sites, *C. sanguinea* subsp. *sanguinea* occupied semiopen scrubland (S35) habitats and, therefore, produced higher fruit yield than occurring in broadleaved deciduous forest habitats (T1B and T1E). At the Šiauliai and Maišiagala sites, stands of *C. sanguinea* subsp. *australis* were comparatively young and, therefore, produced fewer seeds, though the density and coverage of individuals were almost the same as in *C. sanguinea* subsp. *sanguinea* stands.

Data on the density of the soil seed bank of the *Cornus* species are very limited, making it difficult to compare the data from this study with data from other regions. For example, according to the studies performed in Germany, the density of *Cornus sanguinea* subsp. *sanguinea* seeds in the soil range from 10 to 12 seeds m^{-2} in young mixed scrublands and from 64 to 320 seeds m^{-2} in mature scrublands and pine stand [27]. However, another study in Germany [5] has found a much higher seed density of intact seeds (1345 seeds m^{-2}), which is significantly higher than what we found in Lithuania. Thus, the seed density of *Cornus sanguinea* subsp. *sanguinea* found in this study in most cases is considerably higher than those found in Germany. Still, the available information suggests that the density of the seed bank is strongly influenced by habitat conditions and characteristics of the stand. Unfortunately, we cannot make broader comparisons of the soil seed bank density formed by this subspecies and the other *Cornus* taxa studied, as we have not found such published data.

4.3. Viable Seeds

The highest density of intact seeds and the density of viable seeds were found at *C. sanguinea* subsp. *australis* sites; however, no significant difference was found between the density of viable seeds at its sites and sites of *C. alba*. The soil seed bank of these taxa contained significantly more viable seeds than alien *C. sericea* and native *C. sanguinea* subsp. *sanguinea*. It should be noted that both the lowest total seed bank density and the lowest density of viable seeds were recorded at the sites of *C. sericea*. Furthermore, the percentage of viable seeds of *C. sericea* at the individual study sites ranged from 11.8% to 100%, although the mean density of seeds in the soil was generally low.

Studies on *C. sericea* in Ireland have revealed a low seed set and absence of seedlings in the invaded habitats [41]. The study's author supposed that the vegetative spread of *C. sericea* is the main means of reproduction in the invaded areas. We also suppose that seedlings of *C. sericea* play a secondary role in the renewal of its stands. In its native distribution area, the reported mean seed density of *C. sericea* in forest soil samples was low and comprised 26.1 seeds m^{-2}. In addition, it is unclear whether the seeds in the seed bank were viable as they did not germinate under the experimental conditions [56]. Published data on the seed density and viability of *C. sericea* in the seed bank in the invaded range are absent.

The results of studies on seed viability of the other *Cornus* taxa are also quite challenging to interpret unambiguously, as the viability of the seeds was assessed by their germination rate. Studies performed in Germany on the viability of *C. sanguinea* subsp. *sanguinea* seeds extracted from the seed bank have revealed that only 0.89% of all intact seeds germinate. This study showed that the density of germinating seeds was 12 seeds m^{-2}, in contrast to a total density of intact seeds of 1345 seeds m^{-2} [5]. Other studies assessing the germination rate of *C. sanguinea* subsp. *sanguinea* seeds have shown contrasting results. The germination rate of its seeds is 81.0% in a garden experiment. In contrast, in a different study conducted in Germany, the proportion of viable seeds ranged from 53.0% to 73.0% [27]. An experiment performed by Takos and Efthimiou [31] found a germination rate of 65.0% in the laboratory and 79.0% in field sowing. Germination of fresh *C. sanguinea* seeds of summer sowing was found to be 85.8% and 90.8% of autumn sowing [32]. The germination rate and viability of seeds extracted from the soil seed bank may differ significantly from the germination rate and viability of freshly collected seeds, as some of the seeds in the soil may be dormant or may have already lost germination because of age [22,57]. In addition, some authors have suggested that germination rates of *C. sericea* seeds, and possibly seeds of the other related *Cornus* species, depend on precipitation during seed formation and the origin of the sampled population. In the native distribution area of *C. sericea*, the germination rates of its seeds varied from 0–2% to 29–31% in 1984 and from 83–91% to 86–91% in the following year [58].

We found that only 19.4% of *C. sanguinea* subsp. *sanguinea* seeds extracted from the upper soil layer and 18.2% from the lower soil layer were viable. Thus, the seed viability rate was significantly lower than in other studied taxa and substantially lower than reported viability in other studies [5,27,31].

The seed bank of fleshy-fruited plants, including most *Cornus* species, is usually classified as transient or short-lived persistent [8,27]. However, some species prefer forest gaps, including representatives of the genus *Cornus*, forming extensive and long-term seed banks [13,59]. It has been found that seeds of *C. controversa* remain viable for more than ten years [13]. Thus, it cannot be excluded that the different seed viability observed in our study is also due to the different seed longevity of the taxa studied. Although seeds of *C. sanguinea* subsp. *sanguinea* are thought to remain viable for only one or two years and form transient seed banks [27], some studies suggest that they form a short-lived persistent seed bank [28]. As the soil seed bank of the studied *Cornus* taxa has a significant proportion of damaged and non-viable seeds, this ratio confirms the existence of an equilibrium between seed input into the soil seed bank and its depletion. The results of our study support the suggestion by other authors that they form a short-term persistent seed bank [24,26,27]. We, therefore, believe that the longevity, viability and germinability of the *Cornus* seeds should be tested in long-term seed burial experiments.

This research showed that the density of the seed bank formed by the studied *Cornus* taxa varied considerably between the study sites. Still, we also found significant differences in the density of seed banks between taxa. This study supported our hypothesis that most alien *Cornus* taxa form denser soil seed banks than native *C. sanguinea* subsp. *sanguinea*. We found that the seed bank of alien *C. alba*, *C. sericea* and *C. sanguinea* subsp. *australis* contained more viable seeds than the seed bank of native *C. sanguinea* subsp. *sanguinea*. We suppose that high density in a seed bank, especially when made up of viable seeds in the soil, contributes to the rapid spread of alien *Cornus* taxa in the southern part of the Hemiboreal zone of Europe.

Author Contributions: Methodology, L.P., V.R. and Z.G.; investigation, L.P. and Z.G.; data curation, L.P. and D.U.; writing-original draft preparation, L.P. and Z.G.; writing-review and editing, L.P., V.R., D.U. and Z.G.; visualisation, L.P., Z.G. and D.U.; supervision, V.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy concerns.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Richardson, D.M.; Kluge, R.L. Seed banks of invasive Australian *Acacia* species in South Africa: Role in invasiveness and options for management. *Perspect. Plant Ecol. Evol. Syst.* **2008**, *10*, 161–177. [CrossRef]
2. Berge, G.; Hestmark, G. Composition of seed banks of roadsides, stream verges and agricultural fields in southern Norway. *Ann. Bot. Fennici* **1997**, *34*, 77–90.
3. Thompson, K.; Bakker, J.P.; Bekker, R.M. *The Soil Seed Banks of North West Europe: Methodology, Density and Longevity*; Cambridge University Press: Cambridge, UK, 1997.
4. Baskin, C.; Baskin, J.M. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*; Academic Press: San Diego, CA, USA, 1998.
5. Beismann, H.; Kollmann, J.; Bogenrieder, A.; Speck, T. Reconstruction of local vegetation patterns by seed bank analysis-results from three case studies in the Upper Rhine region, Germany. *Neues Jahrb. Geol. Palaontol. Abh.* **1996**, *202*, 169–181. [CrossRef]
6. Faist, A.M.; Ferrenberg, S.; Collinge, S.K. Banking on the past: Seed banks as a reservoir for rare and native species in restored vernal pools. *AoB PLANTS* **2013**, *5*, 043. [CrossRef]
7. Thompson, K.; Grime, J.P. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *J. Ecol.* **1979**, *67*, 893–921. [CrossRef]
8. Walck, J.L.; Baskin, J.M.; Baskin, C.C.; Hidayati, S.N. Defining transient and persistent seed banks in species with pronounced seasonal dormancy and germination patterns. *Seed Sci. Res.* **2005**, *15*, 189–196. [CrossRef]
9. Louda, S.M. Predation in the dynamics of seed regeneration. In *Ecology of Soil Seed Banks*, 1st ed.; Leck, M.A., Parker, V.T., Simpson, R.L., Eds.; Academic Press: London, UK, 1989; pp. 25–51, ISBN 978-0-3231-4865-8.
10. Bekker, R.M.; Bakker, J.P.; Grandin, U.; Kalamees, R.; Milberg, P.; Poschlod, P.; Thompson, K.; Willems, J.H. Seed size, shape and vertical distribution in the soil: Indicators of seed longevity. *Funct. Ecol.* **1998**, *12*, 834–842. [CrossRef]
11. Gioria, M.; Pyšek, P.; Moravcová, L. Soil seed banks in plant invasions: Promoting species invasiveness and long-term impact on plant community dynamics. *Preslia* **2012**, *84*, 327–350.
12. Gioria, M.; Jarošík, V.; Pyšek, P. Impact of alien invasive plants on soil seed bank communities: Emerging patterns. *Perspect. Plant Ecol. Evol. Syst.* **2014**, *16*, 132–142. [CrossRef]
13. Masaki, T.; Tanaka, H.; Shibata, M.; Nakashizuka, T. The seed bank dynamics of *Cornus controversa* and their role in regeneration. *Seed Sci. Res.* **1998**, *8*, 53–63. [CrossRef]
14. Phillips, M.L.; Murray, B.R. Invasiveness in exotic plant species is linked to high seed survival in the soil. *Evol. Ecol. Res.* **2012**, *14*, 83–94.
15. Wearne, L.J.; Morgan, J.W. Shrub invasion into subalpine vegetation: Implications for restoration of the native ecosystem. *Plant Ecol.* **2006**, *183*, 361–376. [CrossRef]
16. Herrera, A.M.; Carruthers, R.I.; Mills, N.J. Introduced populations of *Genista monspessulana* (French broom) are more dense and produce a greater seed rain in California, USA, than native populations in the Mediterranean Basin of Europe. *Biol. Invasions* **2011**, *13*, 369–380. [CrossRef]
17. Goets, S.A.; Kraaij, T.; Little, K.M. Seed bank and growth comparisons of native (*Virgilia divaricata*) and invasive alien (*Acacia mearnsii* and *A. melanoxylon*) plants: Implications for conservation. *PeerJ* **2018**, *6*, e5466. [CrossRef]
18. Archibold, O.W.; Brooks, D.; Delanoy, L. An investigation of the invasive shrub European Buckthorn, *Rhamnus cathartica* L., near Saskatoon, Saskatchewan. *Can. Field-Nat.* **1997**, *111*, 617–621.
19. Giantomasi, A.; Tecco, P.A.; Funes, G.; Gurvich, D.E.; Cabido, M. Canopy effects of the invasive shrub *Pyracantha angustifolia* on seed bank composition, richness and density in a montane shrubland (Córdoba, Argentina). *Austral Ecol.* **2008**, *33*, 68–77. [CrossRef]
20. Gioria, M.; Pyšek, P.; Baskin, C.C.; Carta, A. Phylogenetic relatedness mediates persistence and density of soil seed banks. *J. Ecol.* **2020**, *108*, 2121–2131. [CrossRef]
21. Gioria, M.; Le Roux, J.J.; Hirsch, H.; Moravcová, L.; Pyšek, P. Characteristics of the soil seed bank of invasive and non-invasive plants in their native and alien distribution range. *Biol. Invasions* **2019**, *21*, 2313–2332. [CrossRef]
22. Chambers, J.C.; MacMahon, J.A. A day in the life of a seed: Movements and fates of seeds and their implications for natural and managed systems. *Annu. Rev. Ecol. Syst.* **1994**, *25*, 263–292. [CrossRef]
23. Chee-Sanford, J.C.; Williams, M.M.; Davis, A.S.; Sims, G.K. Do microorganisms influence seed-bank dynamics? *Weed Sci.* **2006**, *54*, 575–587. [CrossRef]
24. Moravcová, L.; Gudžinskas, Z.; Pyšek, P.; Pergl, J.; Perglová, I. Seed ecology of *Heracleum mantegazzianum* and *H. sosnowskyi,* two invasive species with different distributions in Europe. In *Ecology and Management of Giant Hogweed (Heracleum mantegazzianum)*; Pyšek, P., Cock, M.J.W., Nentwig, W., Ravn, H.P., Eds.; CABI: Wallingford, UK; Cambridge, MA, USA, 2007; pp. 157–169.
25. Taura, L.; Kamaitytė-Bukelskienė, L.; Sinkevičienė, Z.; Gudžinskas, Z. Study on the Rare Semiaquatic Plant *Elatine hydropiper* (Elatinaceae) in Lithuania: Population Density, Seed Bank and Conservation Challenges. *Front. Biosci. Landmark* **2022**, *27*, 162. [CrossRef] [PubMed]
26. Van Clef, M.; Stiles, E.W. Stiles. Seed longevity in three pairs of native and non-native congeners: Assessing invasive potential. *Northeast. Nat.* **2001**, *8*, 301–310. [CrossRef]
27. Kollman, J.; Grubb, P.J. Biological flora of Central Europe: *Cornus sanguinea* L. *Flora* **2001**, *196*, 161–179. [CrossRef]

28. Wawrzyniak, M.; Michalak, M.; Chmielarz, P. Effect of different conditions of storage on seed viability and seedling growth of six European wild fruit woody plants. *Ann. For. Sci.* **2020**, *77*, 58. [CrossRef]
29. Bomanowska, A.; Adamowski, W.; Kirpluk, I.; Otręba, A.; Rewicz, A. Invasive alien plants in Polish national parks—Threats to species diversity. *PeerJ* **2019**, *7*, e8034. [CrossRef]
30. Gudžinskas, Z.; Petrulaitis, L. New alien taxa of the genus *Cornus* (Cornaceae) recorded in Lithuania and Latvia. *Botanica* **2021**, *27*, 160–169. [CrossRef]
31. Takos, I.; Efthimiou, G. Germination results on dormant seeds of fifteen tree species autumn sown in a northern Greek nursery. *Silvae Genet.* **2003**, *52*, 67–70.
32. Pipinis, E.; Milios, E.; Mavrokordopoulou, O.; Smiris, P. Effect of sowing date on seedling emergence of species with seeds enclosed in a stony endocarp. *J. Sustain. For.* **2018**, *37*, 375–388. [CrossRef]
33. Ball, P.W. *Cornus L. Flora Europaea: Rosaceae to Umbelliferae*; Tutin, T.G., Heywood, V.H., Burges, N.H., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A., Eds.; Cambridge University Press: Cambridge, UK, 1968; Volume 2, pp. 313–314, ISBN 978-0-5210-6662-4.
34. Gudžinskas, Z.; Petrulaitis, L.; Žalneravičius, E. New woody alien plant species recorded in Lithuania. *Bot. Lith.* **2017**, *23*, 153–168. [CrossRef]
35. Wangerin, W. Cornaceae. In *Das Pflanzenreich. Series IV, Family 229 (Heft 41)*; Engler, H.G.A., Ed.; W. Engelmann: Leipzig, Germany, 1910; pp. 1–101.
36. Zieliński, J.; Tomaszewski, D.; Gawlak, M.; Orlova, L. Kłopotliwe derenie–*Cornus alba* L. i *C. sericea* L. (Cornaceae). Dwa gatunki czy jeden? *Roc. Pol. Tow. Dendrol.* **2014**, *62*, 9–23.
37. Murell, Z.E.; Poindexter, D.B. Cornaceae Bechtold & J. Presl. In *Flora of North America North of Mexico, Magnoliophyta: Vitaceae to Garryaceae*; Flora of North America Editorial Committee, Ed.; Oxford University Press: New York, NY, USA, 2016; Volume 12, pp. 443–457, ISBN 978-0-1906-4372-0.
38. Lindelof, K.; Lindo, J.A.; Zhou, W.; Ji, X.; Xiang, Q.Y. Phylogenomics, biogeography, and evolution of the blue-or white-fruited dogwoods (*Cornus*)—Insights into morphological and ecological niche divergence following intercontinental geographic isolation. *J. Syst. Evol.* **2020**, *58*, 604–645. [CrossRef]
39. Woźnicka, A.; Melosik, I.; Morozowska, M. Quantitative and qualitative differences in morphological traits of endocarps revealed between *Cornus* L. species. *Plant Syst. Evol.* **2015**, *301*, 291–308. [CrossRef]
40. Riebl, R.; Meve, U.; Aas, G. Morphologische Variabilität und taxonomische Differenzierung von *Cornus sanguinea*: Nordbayerische Naturstandorte und Strassenbegleitgrün im Vergleich. *Ber. Bayer. Bot. Ges. Erforsch. Heim. Flora* **2017**, *87*, 39–54.
41. Kelly, D.L. *Cornus sericea* L. in Ireland: An incipient weed of wetlands. *Watsonia* **1990**, *18*, 33–36.
42. Chytrý, M.; Tichý, L.; Hennekens, S.M.; Knollová, I.; Janssen, J.A.M.; Rodwell, J.S.; Peterka, T.; Marcenò, C.; Landucci, F.; Danihelka, J.; et al. EUNIS Habitat Classification: Expert system, characteristic species combinations and distribution maps of European habitats. *Appl. Veg. Sci.* **2020**, *23*, 648–675. [CrossRef]
43. Csontos, P. Seed banks: Ecological definitions and sampling considerations. *Commun. Ecol.* **2007**, *8*, 75–85. [CrossRef]
44. Starfinger, U.; Karrer, G. A standard protocol for testing viability with the Triphenyl Tetrazolium Chloride (TTC) Test. *Jul. Kuehn-Arch.* **2016**, *255*, 65–66. [CrossRef]
45. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 9.
46. R Core Team. *R: A Language and Environment for Statistical Computing*; R Fondation for Statistical Computing: Vienna, Austria. Available online: https://www.R-project.org/ (accessed on 19 April 2022).
47. Fournier, D.A.; Skaug, H.J.; Ancheta, J.; Ianelli, J.; Magnusson, A.; Maunder, M.N.; Nielsen, A.; Sibert, J. AD Model Builder: Using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim. Methods Softw.* **2012**, *27*, 233–249. [CrossRef]
48. Pérez-Fernández, A.M.; Gómez-Gutiérrez, J.M.; Martín-Berrocoso, A.; Reinier, M. Effect of seed shape and size on their distribution in the soil seed bank. *J. Mediterr. Ecol.* **2002**, *3*, 11–17.
49. Martínez-Ghersa, M.A.; Ghersa, C.M. The relationship of propagule pressure to invasion potential in plants. *Euphytica* **2006**, *148*, 87–96. [CrossRef]
50. Wall, S.B.V. How rodents smell buried seeds: A model based on the behavior of pesticides in soil. *J. Mammal.* **2003**, *84*, 1089–1099. [CrossRef]
51. Bojňanský, V.; Fargašová, A. *Atlas of Seeds and Fruits of Central and East-European Flora*; Springer: Dordrecht, The Netherlands, 2007; pp. 439–441, ISBN 978-9-4017-7670-7.
52. Thompson, J.N.; Pellmyr, O. Origins of variance in seed number and mass: Interaction of sex expression and herbivory in *Lomatium salmoniflorum*. *Oecologia* **1989**, *79*, 395–402. [CrossRef] [PubMed]
53. Konarska, A. The biology of flowering and structure of selected elements of *Cornus alba* L. flowers. *Acta Agrobot.* **2009**, *62*, 9–15. [CrossRef]
54. Warzecha, B.; Parker, V.T. Differential post-dispersal seed predation drives chaparral seed bank dynamics. *Plant Ecol.* **2014**, *215*, 1313–1322. [CrossRef]
55. Smith, S.B.; DeSando, S.A.; Pagano, T. The value of native and invasive fruit-bearing shrubs for migrating songbirds. *Northeast Nat.* **2013**, *20*, 171–184. [CrossRef]

56. Brown, D. Estimating the composition of a forest seed bank: A comparison of the seed extraction and seedling emergence methods. *Can. J. Bot.* **1992**, *70*, 1603–1612. [CrossRef]
57. Lennon, J.T.; Den Hollander, F.; Wilke-Berenguer, M.; Blath, J. Principles of seed banks and the emergence of complexity from dormancy. *Nat. Commun.* **2021**, *12*, 4807. [CrossRef]
58. Acharya, S.N.; Chu, C.B.; Hermesh, R.; Schaalje, G.B. Factors affecting red-osier dogwood seed germination. *Can. J. Bot.* **1992**, *70*, 1012–1016. [CrossRef]
59. Kollmann, J. Hypotheses on the regeneration niche of fleshy-fruited species in natural forest gaps and edges in central Europe. *Verh. Ges. Ökol.* **1997**, *27*, 85–91.