

Article

Exposure to Cattle Slurry of Different Concentrations Influence Germination and Initial Growth of Selected Grass and Legume Species

Anastazija Gselman ^{1,*} , Vilma Sem ²  and Silva Grobelnik Mlakar ³

¹ Department of Grassland and Fodder Production, Faculty of Agriculture and Life Sciences, University of Maribor, Pivola 10, 2311 Hoče, Slovenia

² Department of Mathematical Methods, Informatics and Statistics in Agriculture, Faculty of Agriculture and Life Sciences, University of Maribor, Pivola 10, 2311 Hoče, Slovenia

³ Department of Organic Agriculture, Crops, Vegetables and Ornamentals, Faculty of Agriculture and Life Sciences, University of Maribor, Pivola 10, 2311 Hoče, Slovenia

* Correspondence: anastazija.gselman@um.si; Tel.: +386-2-320-9035

Abstract: In addition to improving soil quality, the fertilisation of grassland with cattle slurry is often associated with seed dispersal. Most studies focus on the effects of cattle slurry on the germination and early development of weed species, but less is known about how slurry affects the germination process of grasses and forage legumes. The aim of Experiment I of our study was therefore to investigate the influence of soaking time in cattle slurry of different concentrations on *Lolium multiflorum*, *Dactylis glomerata*, *Trifolium pratense* and *Trifolium repens*. Seeds were soaked in undiluted (100%) and diluted cattle slurries (50% and 25%) for 14, 28, 42, 56, 70 and 84 days. Experiment II was conducted to study the initial growth of studied plants from seeds soaked in cattle slurry of different concentrations for 14 days. After the germination test, which was carried out under controlled conditions, the germination index (GI) was calculated. The results (Experiment I) show that a short soaking in cattle slurry (14 days) has no negative effect on the germination process for all species. However, a longer soaking resulted in significantly reduced and delayed germination, especially in undiluted slurry for grasses and diluted slurries for clovers. The slurry concentration (Experiment II) only influenced the root growth of *L. multiflorum*. Seedlings grown from seeds soaked in undiluted slurry had a 17% higher relative root length than the control and developed significantly longer root systems than the other two slurry concentrations.

Keywords: cattle slurry; soaking time; seed germination; initial plant growth; *Lolium multiflorum*; *Dactylis glomerata*; *Trifolium pratense*; *Trifolium repens*



Citation: Gselman, A.; Sem, V.; Grobelnik Mlakar, S. Exposure to Cattle Slurry of Different Concentrations Influence Germination and Initial Growth of Selected Grass and Legume Species. *Agriculture* **2023**, *13*, 1999. <https://doi.org/10.3390/agriculture13101999>

Academic Editor: Hilary Mayton

Received: 1 September 2023

Revised: 29 September 2023

Accepted: 13 October 2023

Published: 15 October 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Semi-natural grassland covers a large part of the earth's surface and about 30% of the agricultural land in Europe. It serves as a source of fodder for livestock, a habitat for wildlife, environmental protection (e.g., carbon sequestration and soil conservation) and in situ conservation of plant genetic resources [1]. There are still large areas of semi-natural grassland in both Eastern Europe (Romania and Poland together have about two million hectares) and Slovenia (53.6% of semi-natural grassland is within the total agricultural area). Many livestock farmers in Slovenia and elsewhere depend on grassland and its products such as hay and silage [2].

Intensive grassland management (widespread use of herbicides, mineral fertilisers, frequent cutting regime and reseeding of swards) improves fodder production and its quality [3–5], but on the other hand often leads to the disappearance of traditional land uses (such as mowing with grazing in late autumn) and reduces plant diversity [4,6,7]. It is clear from the literature [8–10] that the seed bank often lacks desirable target species

during prolonged periods of intensification or abandonment. Therefore, the European Union (EU) has committed to planning for the restoration of biodiversity, including natural grasslands, through its Biodiversity Strategy 2030 and the Green Deal Initiative. The aim of these strategies is to halt biodiversity loss and achieve sustainable growth, as well as to maintain and/or enhance grassland biodiversity in EU Member States. The focus is on revitalising degraded habitats and expanding the network of protected areas (PAs: NATURA 2000) [11,12].

It is well known that seasonally late mowing of hay allows seed dispersal of plant species and has a positive effect on grassland biodiversity, e.g., on the size of the seed bank in the soil and on species diversity [13–15], as up to 95% of the plants are mature at the traditionally late time of hay mowing (mid-August) and this value increases to 100% if mowing is done even later [16]. Natural recolonization can also occur through seed dispersal by applied organic manure (e.g., slurry) or grazing livestock [17,18], but this process is usually slow and unreliable, even under favourable conditions, and often results in communities with lower floristic diversity [19]. Therefore, active introduction of species is usually required [20,21].

In recent years, numerous management methods have been introduced to maintain species-rich grasslands or to improve the botanical diversity of intensively managed grasslands [22]. The most commonly practised method is soil fertilisation. It can be generally assumed that reducing the availability of nutrients in the soil increases the richness of plant species [23,24]. However, Chytrý et al. [25] reported that appropriate fertilisation can actually increase species richness in species-poor grassland with low productivity.

Traditional haymaking is another practise that contributes to the conservation of the biotic diversity of the grasslands. Typically, hay seeds were collected from the barns where the hay was stored and then distributed to the grasslands [26,27]. Nowadays, modern cattle production systems often include pasture-based milk and beef production, where winter stabling usually lasts about six months, depending on the system, location and soil type. The animals also receive a proportion of hay in their feed ration. This means that the seeds from the feeding table also end up in the cattle's slurry [28].

About 80% of the slurry produced in winter stables is spread as slurry and used as fertiliser on semi-natural grassland, especially on livestock farms and mixed farms. It is also typical that on farms where most of the forage is used during the stable season, the slurry is applied back to the land from which the winter forage was harvested [28]. It is therefore possible that the application of cattle slurry not only contributes to the dispersal of plant seeds, but that the application of cattle slurry can also lead to the locally favoured establishment of seedlings in the meadow [29–31]. This approach can contribute to longer sward life and is an added benefit, as research by Volden [32] has shown, since slurry has minimal negative effects on grass and clover seed germination. In a study by Jones and Roberts [33], who investigated the maintenance of annual ryegrass, slurry seeding was found to be effective in extending the sward life of the species.

While there are numerous studies and methodologies addressing seed dispersal mechanisms and strategies to promote botanical diversity in grassland ecosystems, comprehensive data on the dynamics of seed germination and its intricate relationship with the temporal exposure of seeds to cattle slurry are still lacking. Cattle slurry is a commonly used organic fertiliser as well as seed dispersal medium that could affect seed survival, germination process and early growth of plant species. Since most previous research in this field focused on weeds, the economically important grasses and legumes that are also found in slurry have been overlooked. The main objective of this study was to determine the effects of different concentrations and soaking times of cattle slurry on the germination and initial growth of certain grassland plant species (including two grass species: *Lolium multiflorum* Lam. and *Dactylis glomerata* L., and two legume species: *Trifolium pratense* L. and *Trifolium repens* L.), with implications for maintaining biodiversity in natural grassland ecosystems. Achieving these objectives should also provide valuable insights into the potential benefits

and challenges associated with the use of cattle slurry as a medium for seed dispersal and thus for biodiversity conservation in the fragile ecosystems of natural grasslands.

2. Materials and Methods

Cattle slurry from a moderately intensive dairy farm was used for the experiment. Well-mixed slurry stored for six months was taken from the slurry pit and filled into the plastic containers. The samples were diluted with tap water to three concentrations: 100% concentration (undiluted slurry; 10 L slurry; 11.8% dry matter (DM) and 836 g organic matter (OM)/kg DM), 50% concentration (5 L slurry + 5 L water; 4.7% DM and 331 g OM/kg DM) and 25% concentration (2.5 L slurry + 7.5 L water; 1.9% DM and 205 g OM/kg DM). The pH value of the undiluted cattle slurry was 7.4.

The certified seed of two forage grasses—annual ryegrass (*L. multiflorum*) ‘Melquatro’, cocksfoot (*D. glomerata*) ‘Kopa’ and two legume species—white clover (*T. repens*) ‘Grasslands huia’ and red clover (*T. pratense*) ‘Global’ (all provided by Semenarna Ljubljana, Slovenia), were placed in permeable polypropylene bags made of plant cover fabric (Vrteks; produced by the sanitary material factory TOSAMA, Slovenia). Each bag contained four smaller bags filled with about 100 g of seeds. The bags of seeds were soaked in each of the three concentrations of cattle slurry for 14, 28, 42, 56, 70 and 84 days.

2.1. Experiment I: Germination of Forage Grasses and Legumes

After a specified soaking time, 30 seeds from each treatment, together with the control (C—untreated seeds), were placed evenly in sterile Petri dishes (9 cm diameter) lined with three layers of filter paper (FT-3-101-090, 0.21 mm). The filter papers were initially moistened with 5 mL distilled water. Petri dishes were arranged in a completely randomised block design in four replications and incubated in a growth chamber (LO 600-S, IZR, Škofja Loka, Slovenia) at a relative humidity of 75%, a 14 h/10 h light/dark period and a temperature regime of $25/15 \pm 1$ °C day/night.

The seed germination process was monitored at 24 h intervals throughout the experiment. A seed was considered viable (germinated) when the radicle visibly expanded and reached a length of approximately 1 mm. At the end of the 18-day experiment, the Final Germination (Gf) was calculated. However, since Gf represents only the final percentage of germination of the seed lot and does not provide information on the speed and uniformity of germination, the Germination Index (GI) was also considered. At GI, viable seeds counted on the first day receive the greatest weight, and viable seeds counted later receive a lesser weight. Therefore, GI emphasizes both the percentage of germination and its speed; a higher GI value means a higher percentage and speed of germination [34].

The Gf and GI indices were calculated according to Equations (1) and (2), respectively:

$$Gf = \text{No. of germinated seeds at final measurement} / 30 \times 100, \quad (1)$$

$$GI = (18 \times N1) + (17 \times N2) + (16 \times N3) + \dots + (1 \times N18), \quad (2)$$

where N1, N2, N3 . . . N18 are the number of viable seeds on the first, second, and following days until the last, 18th day, and the factors 18, 17, 16 . . . and 1 are the weights assigned to the number of viable seeds on the first, second, third and subsequent days, respectively.

2.2. Experiment II: Initial Growth of Forage Grasses and Legumes

For Experiment II, the seeds of the species studied were used, which had been soaked in cattle slurry in permeable polypropylene bags for 14 days. These seeds (30 per replication) were placed in sterile Petri dishes and arranged in a completely randomized block design with four replications. Over a span of 21 days, the seeds were allowed to germinate within a growth chamber under the same conditions as in Experiment I. The resulting plants were then photographed using a Canon SZ61 camera. The length of both the individual seed roots and shoots of each plant was measured using a stereomicroscope (Olympus SZ61, Japan). For the grass species studied, the length of the root system of the young plant

was calculated as the sum of the individual seed root lengths. Similarly, the length of the coleoptile and the first developed leaf were summed and expressed as the shoot length.

The Relative Root Length (RRL) and Relative Shoot Length (RSL) were calculated using Equations (3) and (4):

$$\text{RRL (\%)} = \text{Average root (root system) length in treatment} / \text{Average root (root system) length in control}, \quad (3)$$

$$\text{RSL (\%)} = \text{Average shoot in treatment} / \text{Average shoot length in control}. \quad (4)$$

2.3. Data Analyses

Analyses of variance (ANOVA) of a randomised block design were performed separately for the species-specific data sets using Statgraphics Centurion XV (Statpoint Technologies Inc., Herndon, VA, USA), with the significance level set at $p < 0.05$. Before running ANOVA, certain transformations were applied to the data to improve the normality of distribution and meet the assumption of homogeneity of variance. In particular, the Germination Index data (GI) for all species were subjected to a square root (SQRT) transformation. Relative Root Length (RRL) and Relative Shoot Length (RSL) data were considered untransformed. Data from Experiment I were subjected to a two-factorial ANOVA and data from Experiment II were analysed using a two-way ANOVA. Post-hoc analyses were conducted to compare the means of the main factors' treatments (Duncan test), comparisons of the interaction treatments were made out using the Tukey's test ($\alpha = 0.05$) and Dunnett's test was used to compare treatments with the control. Results are presented as estimated means $\pm$ standard deviation (SD).

3. Results

3.1. Experiment I: Germination of Forage Grasses and Legumes

Although the two grass species had different germination abilities and different germination patterns (Figure 1), seed germination in both species was significantly reduced and delayed by the prolonged soaking and increased concentration of cattle slurry. While seeds of both grasses exposed to undiluted cattle slurry for more than 14 days clearly did not reach 50% of the final germination observed in the respective controls, *L. multiflorum* showed comparatively less susceptibility to prolonged soaking, lasting up to one month in diluted slurry, compared to *D. glomerata*. However, the seeds of *D. glomerata* soaked in 25% diluted slurry germinated faster and achieved higher final germination than the control.

Kader [34] showed that the GI is one of the most comprehensive germination indices. It combines the percentage of germination and the speed of the germination process and thus improves the visibility of variation between seed lots by providing easily comparable numerical data. For this reason, ANOVA was applied exclusively to GI.

For *L. multiflorum* and *D. glomerata*, the ANOVA (Table 1) showed highly significant effects ($p < 0.001$) of soaking time on GI (F-value of 96.9 and 151.1, respectively), together with the influence of cattle slurry concentration (F-value of 17.2 and 5.0, respectively). In addition, a significant interaction was observed between the factors studied (F-value of 2.7 and 6.0 for *L. multiflorum* and *D. glomerata*, respectively).

The results clearly show that prolonged soaking has a detrimental effect on the GI of *L. multiflorum* (Table 1). While the values of GI after 14 days of soaking (423, 355, and 359 for 100%, 50%, and 25% slurry concentration, respectively) were comparable to those of the control (438), a longer soaking time resulted in a significant decrease in GI. The decrease was most pronounced in undiluted slurry, where GI dropped to 109 or 25% of the control value after only 28 days of soaking. Averaged across all concentrations, GI gradually declined from 379 in the 14-day soaking treatments to only 10 when seeds were soaked for three months (84 days). On average, the undiluted slurry had a more deleterious effect on the GI of *L. multiflorum* (108) than the 50% and 25% diluted slurry (159 and 137, respectively).

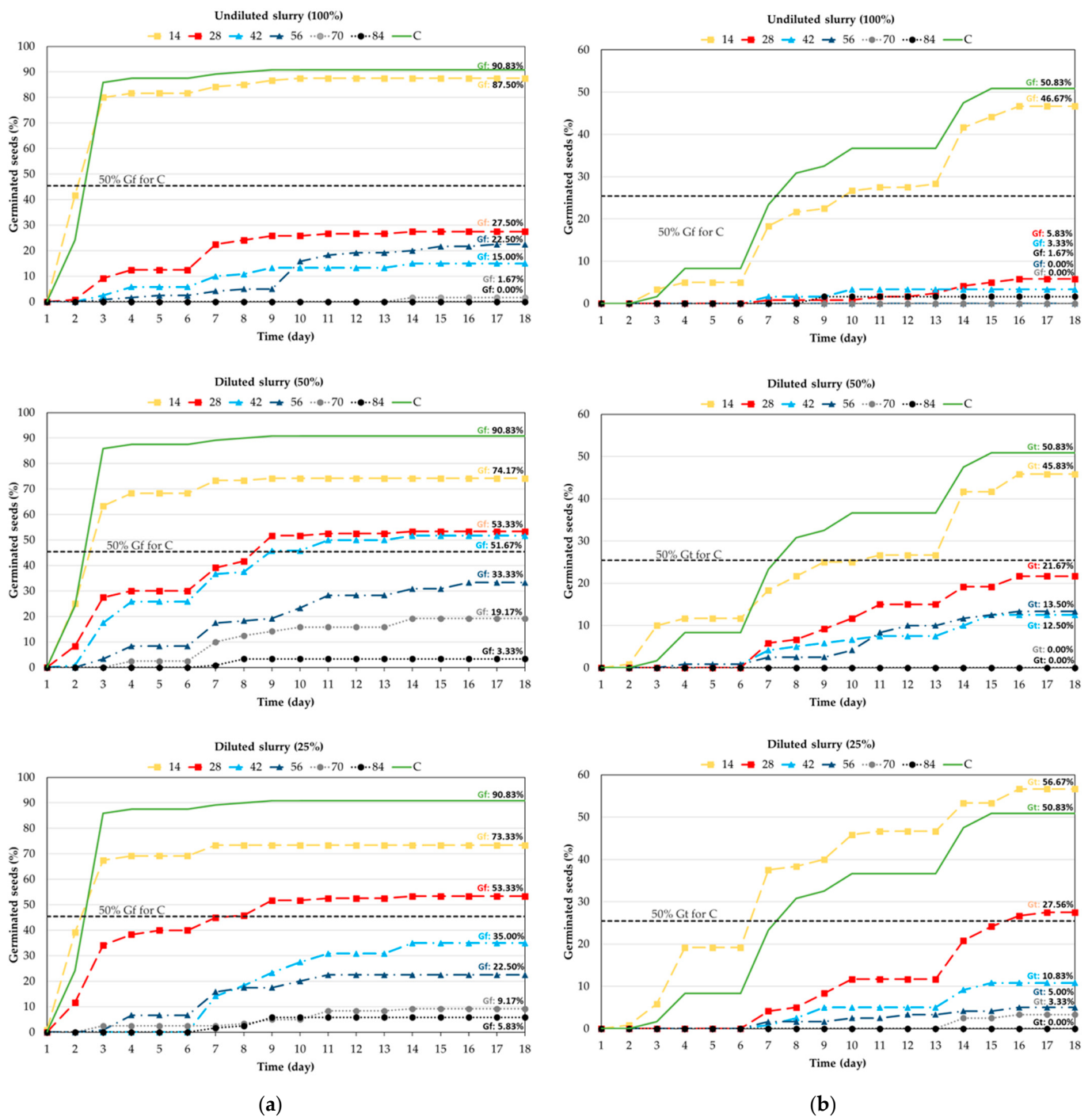


Figure 1. Dynamics of seed germination in: (a) *L. multiflorum* and (b) *D. glomerata* as a function of soaking time in cattle slurry of different concentrations.

The same gradual and even steeper decline of GI was also observed in *D. glomerata* (Table 1). On average, the values of GI dropped from 150 (after 14 days of soaking) to only 2 when the seeds were soaked for more than two months. Examining the interaction and comparing treatments where seeds were soaked for 14 days, we found that GI was statistically equal to the control (153) only in seeds soaked with slurry diluted to 50% (131). In contrast, GI was lower in seeds soaked in undiluted slurry (125) and significantly higher than control when seeds were soaked in 25% diluted cattle slurry (193). As with *L. multiflorum*, GI was also lower for *D. glomerata* when seeds were soaked in 100% slurry (25)

compared with diluted slurries where GI was estimated to be 42 and 48 at 50% and 25% concentrations, respectively.

Table 1. Effect of soaking time in cattle slurry of different concentrations on the Germination Index (GI) of the grasses studied.

Soaking Time (Days)	<i>L. multiflorum</i>				<i>D. glomerata</i>			
	Cattle Slurry Concentration (%)				Cattle Slurry Concentration (%)			
	100	50	25	Mean	100	50	25	Mean
14	423 ± 40.4 a*	355 ± 56.8 a*	359 ± 57.1 a*	379 A	125 ± 26.4 b	131 ± 8.5 b*	193 ± 33.2 a	150 A
28	109 ± 21.1 cd	220 ± 78.3 b	228 ± 36.1 b	186 B	11 ± 4.1 d	55 ± 15.8 c	57 ± 6.6 c	41 B
42	55 ± 5.5 d	201 ± 80.9 bc	105 ± 31.0 cd	120 C	11 ± 7.2 d	32 ± 12.5 cd	24 ± 8.8 cd	22 C
56	57 ± 2.9 d	106 ± 22.2 cd	82 ± 28.5 d	82 C	0 ± 0.0 d	32 ± 17.5 cd	12 ± 8.5 d	15 C
70	3 ± 1.9 d	61 ± 16.0 d	29 ± 6.9 d	31 D	0 ± 0.0 d	0 ± 0.0 d	5 ± 1.0 d	2 D
84	0 ± 0.0 d	11 ± 6.0 d	19 ± 8.5 d	10 E	5 ± 10.0 d	0 ± 0.0 d	0 ± 0.0 d	2 D
Mean	108 B	159 A	137 A		25 B	42 A	48 A	
C	438 ± 13.1				153 ± 7.1			

A–E means of the main factors' treatments followed by different letters are significantly different (Duncan, $\alpha = 0.05$). a–d means ($\pm$ SD) of interaction treatments followed by different letters are significantly different (Tukey, $\alpha = 0.05$). * values followed by an asterisk are not significantly different from those of the control (Dunnett, $\alpha = 0.05$).

From Figure 2, which shows the germination dynamics of the two clover species, it can be seen that for both *T. repens* and *T. pratense* there is a marked decrease in the final germination percentage when the soaking time exceeds 14 days. Apart from the treatment in which *T. repens* seeds were soaked in undiluted slurry for 28 days, all other treatments failed to achieve even 50% of the final germination observed in the respective controls.

ANOVA shows that soaking duration had the greatest influence ($p < 0.001$) on the GI of *T. repens* and *T. pratense* (F-value of 113.4 and 110.7, respectively), followed by slurry concentration (F-value of 6.1 and 110.7, respectively). The interaction was significant ($p < 0.001$) and is shown in Table 2.

Table 2. Effect of soaking time in cattle slurry of different concentration on the Germination Index (GI) of the forage legumes studied.

Soaking Time (Days)	<i>T. repens</i>				<i>T. pratense</i>			
	Cattle Slurry Concentration (%)				Cattle Slurry Concentration (%)			
	100	50	25	Mean	100	50	25	Mean
14	428 ± 54.1 a*	379 ± 44.2 a*	370 ± 22.1 a*	392 A	397 ± 30.4 a*	364 ± 28.7 a*	416 ± 17.3 a*	392 A
28	146 ± 37.4 b	23 ± 7.6 c	16 ± 11.4 c	62 B	258 ± 33.0 b	33 ± 5.3 c	20 ± 11.7 c	104 B
42	35 ± 3.7 c	9 ± 5.4 c	35 ± 11.6 c	26 C	10 ± 6.9 c	24 ± 8.3 c	4 ± 0.3 c	12 C
56	14 ± 5.0 c	8 ± 4.6 c	10 ± 5.9 c	11 C	11 ± 6.8 c	10 ± 3.7 c	4 ± 0.4 c	9 C
70	9 ± 5.7 c	23 ± 7.5 c	9 ± 0.9 c	14 C	10 ± 6.1 c	15 ± 9.9 c	15 ± 2.0 c	13 C
84	0 ± 0.0 c	15 ± 5.8 c	0 ± 0.0 c	5 C	16 ± 9.3 c	28 ± 9.7 c	0 ± 0.0 c	15 C
Mean	105 A	76 B	73 B		117 A	79 B	77 B	
C	393 ± 21.5				412 ± 16.7			

A–C means of the main factors' treatments followed by different letters are significantly different (Duncan, $\alpha = 0.05$). a–c means ($\pm$ SD) of interaction treatments followed by different letters are significantly different (Tukey, $\alpha = 0.05$). * values followed by an asterisk are not significantly different from those of the control (Dunnett, $\alpha = 0.05$).

As with the grasses, the GI values of clover seeds soaked in slurries for 14 days (392) was not statistically different from those of the controls (393 for *T. repens* and 412 for *T. pratense*), but values decreased with longer soaking. GI values decreased particularly drastically when soaking time is extended to a period longer than one month. In contrast to the grasses, however, the decline was most pronounced in diluted slurries (Table 2).

In contrast to the forage grasses studied, the germination process of *T. repens* and *T. pratense* was less affected after seed soaking in undiluted slurry (105 and 117, respectively) than in slurries diluted to 50% and 25% (*T. repens*: 76 and 73 and *T. pratense* 79 and 77, respectively).

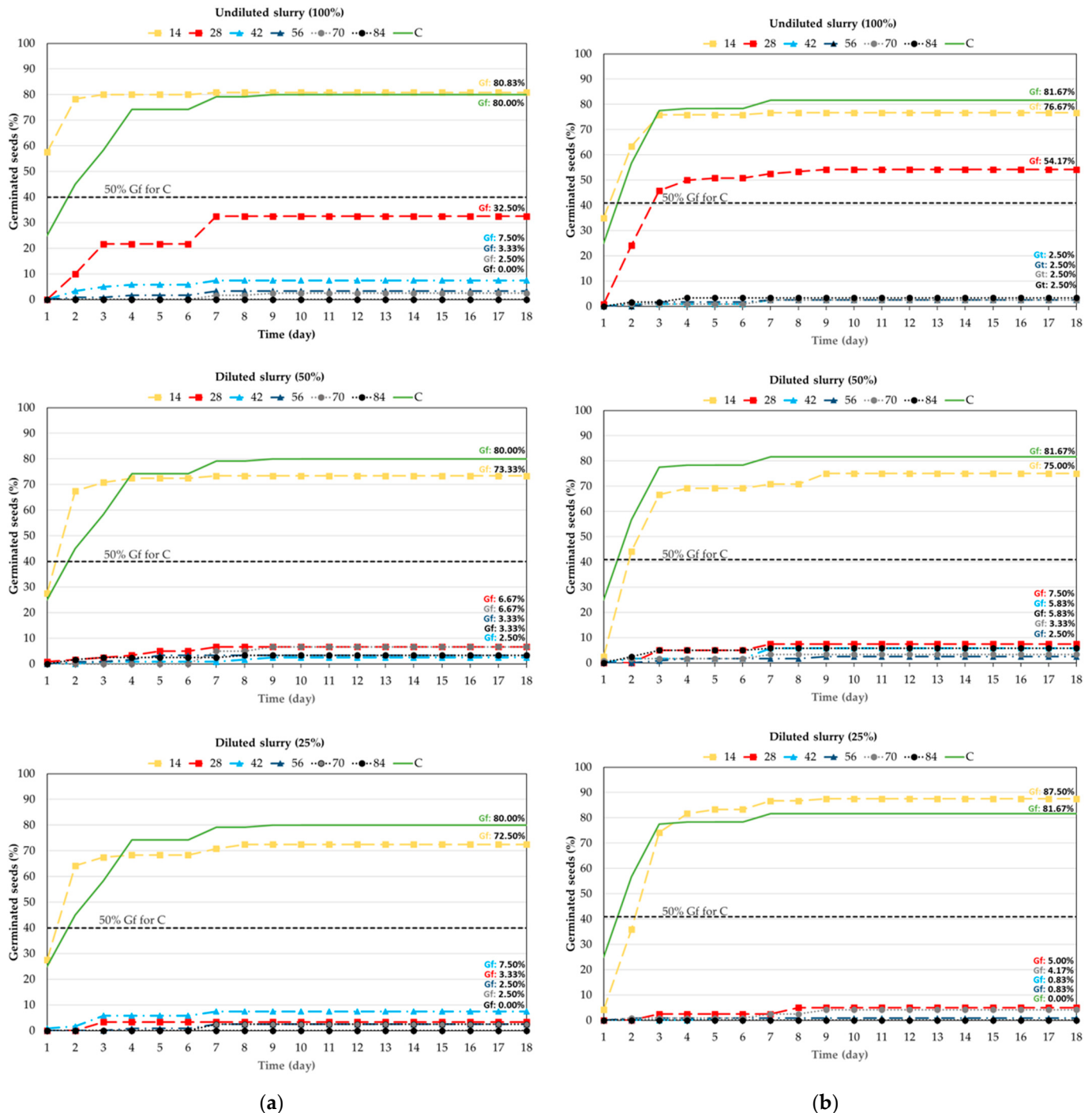


Figure 2. Seed germination dynamics for: (a) *T. repens* and (b) *T. pratense* as a function of soaking time in cattle slurry of different concentrations.

3.2. Experiment II: Initial Growth of Forage Grasses and Legumes

Since soaking the seeds in cattle slurry of different concentrations for 14 days did not show significant differences in most of the plant species studied compared to their respective controls (except for *D. glomerata*), we focused on investigating whether the slurry

concentration affected the early growth of the seedlings. The analysis of the results showed that the slurry concentration only influenced the root growth of *L. multiflorum* (Table 3). Seedlings grown from seed soaked with undiluted slurry had a 17% higher Relative Root Length (RRL) than those of the control (161.6 mm) and developed significantly longer root systems than the other two slurry concentrations tested.

Table 3. Relative Root Length (RRL) and Relative Shoot Length (RSL) of 21-day-old forage grasses and legumes after soaking in different concentrations of cattle slurry.

Slurry Conc. RRL and RSL (%)	<i>L. multiflorum</i>		<i>D. glomerata</i>		<i>T. repens</i>		<i>T. pratense</i>	
	RRL *	RSL ^{ns}	RRL ^{ns}	RSL ^{ns}	RRL ^{ns}	RSL ^{ns}	RRL ^{ns}	RSL ^{ns}
100	117 ± 20.3 a	87 ± 20.0	112 ± 16.5	105 ± 19.7	105 ± 24.1	84 ± 6.1	71 ± 9.8	76 ± 7.6
50	91 ± 4.8 b	96 ± 16.1	100 ± 22.0	96 ± 7.6	87 ± 28.2	77 ± 17.4	95 ± 20.2	66 ± 2.1
25	87 ± 9.3 b	85 ± 9.4	128 ± 20.8	115 ± 20.3	111 ± 12.2	91 ± 20.5	83 ± 13.2	80 ± 10.3
C lengths (mm)	161.6	94.2	58.0	112.1	48.1	16.0	62.9	23.1

* Significant at $p < 0.05$ and non-significant (ns). a,b means ($\pm$ SD) followed by different letters are significantly different (Duncan, $\alpha = 0.05$).

4. Discussion

There are few recent studies on how different concentrations of slurry and different soaking times for seeds in cattle slurry affect the germination and initial growth of certain plant species. While most studies have focused on the effects of treating weed seeds with various organic amendments, including fertilisers of animal origin, on the germination rate of other plant species [35–37], none of these studies have specifically addressed how the duration of soaking grass and legume seeds in cattle slurry affects their germination and initial growth. Nevertheless, previous research has provided valuable insights, some of which are consistent with the results of our own research. An example from the mid-20th century is the pioneering study by Wollan et al. [38], who investigated the effects of sewage sludge on seed germination. The results of their pot experiments investigating the effects of sewage sludge on *Hordeum vulgare* and *Lolium* spp. seed germination showed that germination was inhibited in the presence of sewage sludge. The authors find a strong correlation between the content of OM in the mixtures of sewage sludge and soil in which the seeds were grown and the effects on germination. Comparative tests conducted on mixtures with different contents of OM showed that the delay in germination was most pronounced in the mixtures with higher OM contents, which roughly corresponds to the germination index and initial growth of the plant species in our research (Experiments I and II). It is assumed that when sewage sludge or other liquid fertilisers with high OM concentrations, such as the slurry in our experiments where OM ranged from 836 g OM/kg DM, 331 g OM/kg DM to 205 g OM/kg DM, came into direct contact with plant seeds, increased microbial activity set in, leading to a reduction in oxygen levels and creating an environment conducive to the formation of volatile sprout inhibitors such as ethylene and ammonia, as explained by Wong et al. [39].

Perhaps most similar to our experiments are the results of the study obtained from sowing with slurry [40]. In this method, the seeds of *Raphanus sativus*, *L. multiflorum* and *Avena sativa* are mixed with the slurry in the application tank for a certain time. The results obtained illustrate the effects of slurry on the germination of the above-mentioned plant seeds and their possible consequences if the method of slurry sowing is used. Immersing the seed in the solution (100% slurry or diluting the slurry with water) can influence germination as the seed is soaked and this acts as a trigger for germination. Furthermore, the penetration of water into the seed can transport toxic components that can affect germination, which also happens when the seed enters the slurry from the feed table in the barn. The results showed high variability in the successful establishment and growth of all plants involved, which is also consistent with our results.

The variability in successful initial plant growth could also be due to the leaching of ammonia, urea and other inhibitory substances from the decomposing slurry. Allred and

Ohlrogge [41] already confirmed in their laboratory studies that NH_3 is toxic, especially in the initial phase of germination of *Zea mays* seeds (in the first 2 days), and that volatile (free) NH_3 also affects the emergence of germinated seeds. Consequently, we can assume that the interaction of different components in cattle slurry and the presence of unbound forms of ammonia could be the main factors responsible for the reduced germination rates and subsequent effects on seedlings initial growth.

Similar results to ours were obtained by Suarez [42], whose research aimed to determine the effects of slurry and exposure time on the germination of several plant species (*Triticum aestivum*, *Secale cereale*, *L. multiflorum*, *Avena sativa*, *Trifolium incarnatum*, *Brassica oleracea* convar. *acephala*, *Raphanus sativus* var. *longipinnatus* and *B. campestris*). The author stated that pure slurry (100% concentration) has an inhibitory effect on seed germination in all species. Diluted slurry at 2/3 strength also had an inhibitory effect on germination and behaved very similarly to 100% slurry. Slurry at one-third strength strongly inhibited germination of *T. incarnatum*, but the effect on the other species were less pronounced. Legumes, e.g., *T. incarnatum*, appear to be more susceptible to slurry than grasses and other species, with a sharp reduction in germination at one-third strength (83% reduction compared to estimated total germination and about 79% reduction compared to controls for each species). For the grasses and the other species, the reduction was between 21–29% of the expected total germination and about 10–29% compared to the controls for the same species, which is quite comparable to our study. The results obtained also showed that regardless of the immersion solution (water or slurry), longer immersion periods (≥ 24 h) had a detrimental effect on the germination of the experimental plants. The reduction in germination rate of *R. sativus* var. *longipinnatus* and *A. sativa* was more negative due to this immersion effect. Short immersion ≤ 1 h (also in slurry) showed the highest germination rates for all species ($\geq 70\%$). We therefore assume, which is also confirmed by our investigations, that with increasing soaking time in diluted or undiluted cattle slurry, both the Germination Index and seedling initial growth decrease.

Hoekstra et al. [43] investigated the effects of cattle manure from farms with different feeding strategies (extensive organic, intensive organic, integrated and conventional) on germination and initial root growth of cress (*Lepidium sativum*). Our results are comparable to those of this study, as both showed that seed germination and root growth decreased with higher concentrations of organic fertilisers. Stimulating effects on root growth were observed at concentrations of 1% and 3% [43]. Overall, the study also concluded that manure from farms with different feeding strategies can differ significantly in terms of phytotoxicity (presence of heavy metals). Relative seed germination after 24 h showed significant differences at a concentration of 30% and was most strongly correlated with water-soluble copper. We therefore assume that the Germination Index and early growth (RRL and RSL) of grass and legume seeds could also be influenced by heavy metals, which were not investigated in the experiments we conducted.

5. Conclusions

Survival of forage plant seeds in a cattle slurry has an impact on competition between grassland plants and site dominance, promoting sustainable yields and biodiversity. In line with our research objectives, we found that different concentrations of cattle slurry affect seed germination and early plant growth. As previously described in:

- Experiment I: A short 14-day soaking in cattle slurry has no negative effect on germination of *L. multiflorum*, *D. glomerata*, *T. repens* and *T. pratense*. However, longer soaking (more than 14 days) resulted in reduced and delayed germination of these tested plants, especially in undiluted slurries for grasses and diluted slurries for clovers.
- Experiment II: Soaking seeds in cattle slurry of different concentrations for 14 days only affected the initial growth of *L. multiflorum*. Seedlings of seeds soaked in undiluted slurry have higher RRL than the corresponding control.

Further research in this area is needed as the information could provide valuable insights into the complex relationship between cattle slurry application and seed survival.

However, the information obtained in this study may help to refine the cultivation techniques required to meet the conditions of the EU Biodiversity Strategy and the Green Deal initiative in relation to the conservation of biotic diversity.

Author Contributions: Conceptualization, A.G. and S.G.M.; data curation, A.G. and S.G.M.; formal analysis, S.G.M. and V.S.; investigation, A.G. and S.G.M.; methodology, A.G. and S.G.M.; resources, A.G. and S.G.M.; supervision, A.G., V.S. and S.G.M.; validation, A.G. and S.G.M.; visualization, A.G. and S.G.M.; Writing—original draft, A.G. and S.G.M.; Writing—review and editing, A.G. and S.G.M. 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.

Data Availability Statement: The data presented in this study are available within the article.

Acknowledgments: The authors are very grateful to Rok Brajnik, Sanja Krajnc and Danijela Ačko for their excellent field work and assistance during the laboratory.

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

References

1. Huyghe, C.; De Vlieghe, A.; van Gils, B.; Huyghe, C. *Grasslands and Herbivore Production in Europe and Effects of Common Policies*; Éditions Quæ: Versailles, France, 2014.
2. Veen, P.; Molnár, Z.; Pärtel, M.; Nagy, S. *Grassland Ecosystems in Central and Eastern Europe—Prepared in the Framework of the High Level Conference on EU Enlargement ‘The Relation between Agriculture and Nature Management’—22/24 January 2001*; Veen Ecology: Nunspeet, The Netherlands, 2001.
3. Dauber, J.; Bengtsson, J.A.N.; Lenoir, L. Evaluating Effects of Habitat Loss and Land-Use Continuity on Ant Species Richness in Seminal Grassland Remnants. *Conserv. Biol.* **2006**, *20*, 1150–1160. [[CrossRef](#)]
4. Plantureux, S.; Peeters, A.; McCracken, D. Biodiversity in intensive grasslands: Effect of management, improvement and challenges. *Agron. Res.* **2005**, *3*, 153–164.
5. Walker, K.J.; Stevens, P.A.; Stevens, D.P.; Mountford, J.O.; Manchester, S.J.; Pywell, R.F. The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biol. Conserv.* **2004**, *119*, 1–18. [[CrossRef](#)]
6. Hodgson, J.G.; Montserrat-Martí, G.; Tallowin, J.; Thompson, K.; Díaz, S.; Cabido, M.; Grime, J.P.; Wilson, P.J.; Band, S.R.; Bogard, A.; et al. How much will it cost to save grassland diversity? *Biol. Conserv.* **2005**, *122*, 263–273. [[CrossRef](#)]
7. Hopkins, A.; Holz, B. Grassland for agriculture and nature conservation: Production, quality and multi-functionality. *Agron. Res.* **2006**, *4*, 3–20.
8. Davies, A.; Waite, S. The persistence of calcareous grassland species in the soil seed bank under developing and established scrub. *Plant Ecol.* **1998**, *136*, 27–39. [[CrossRef](#)]
9. Bossuyt, B.; Butaye, J.; Honnay, O. Seed bank composition of open and overgrown calcareous grassland soils—A case study from Southern Belgium. *J. Environ. Manag.* **2006**, *79*, 364–371. [[CrossRef](#)]
10. Fagan, K.C.; Pywell, R.F.; Bullock, J.M.; Marrs, R.H. The seed banks of English lowland calcareous grasslands along a restoration chronosequence. *Plant Ecol.* **2010**, *208*, 199–211. [[CrossRef](#)]
11. Hermoso, V.; Carvalho, S.B.; Giakoumi, S.; Goldsborough, D.; Katsanevakis, S.; Leontiou, S.; Markantonatou, V.; Rumes, B.; Vogiatzakis, I.N.; Yates, K.L. The EU Biodiversity Strategy for 2030: Opportunities and challenges on the path towards biodiversity recovery. *Environ. Sci. Policy* **2022**, *127*, 263–271. [[CrossRef](#)]
12. European Commission Environment, D.-G. *EU Biodiversity Strategy for 2030: Bringing Nature Back into Our Lives*; Publications Office of the European Union: Luxembourg, 2021. [[CrossRef](#)]
13. Kull, K.; Zobel, M. High species richness in an Estonian wooded meadow. *J. Veg. Sci.* **1991**, *2*, 715–718. [[CrossRef](#)]
14. Hansson, M.; Fogelfors, H. Management of a semi-natural grassland; results from a 15-year-old experiment in southern Sweden. *J. Veg. Sci.* **2000**, *11*, 31–38. [[CrossRef](#)]
15. Mitlacher, K.; Poschlod, P.; Rosén, E.; Bakker, J.P. Restoration of wooded meadows—A comparative analysis along a chronosequence on Öland (Sweden). *Appl. Veg. Sci.* **2002**, *5*, 63–73. [[CrossRef](#)]
16. Dahlstrom, A.; Lennartsson, T.; Wissman, J. Biodiversity and traditional land use in South-Central Sweden: The significance of management timing. *Environ. Hist.* **2008**, *14*, 385–403. [[CrossRef](#)]
17. Barbaro, L.; Dutoit, T.; Cozic, P. A six-year experimental restoration of biodiversity by shrub-clearing and grazing in calcareous grasslands of the French Prealps. *Biodivers. Conserv.* **2001**, *10*, 119–135. [[CrossRef](#)]
18. Fagan, K.C.; Pywell, R.F.; Bullock, J.M.; Marrs, R.H. Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. *J. Appl. Ecol.* **2008**, *45*, 1293–1303. [[CrossRef](#)]

19. Stampfli, A.; Zeiter, M. Plant Species Decline Due to Abandonment of Meadows Cannot Easily Be Reversed by Mowing. A Case Study from the Southern Alps. *J. Veg. Sci.* **1999**, *10*, 151–164. [\[CrossRef\]](#)
20. Pywell, R.F.; Bullock, J.M.; Hopkins, A.; Walker, K.J.; Sparks, T.H.; Burke, M.J.W.; Peel, S. Restoration of species-rich grassland on arable land: Assessing the limiting processes using a multi-site experiment. *J. Appl. Ecol.* **2002**, *39*, 294–309. [\[CrossRef\]](#)
21. Pywell, R.F.; Bullock, J.M.; Tallowin, J.B.; Walker, K.J.; Warman, E.A.; Masters, G. Enhancing diversity of species-poor grasslands: An experimental assessment of multiple constraints. *J. Appl. Ecol.* **2007**, *44*, 81–94. [\[CrossRef\]](#)
22. Edwards, A.R.; Mortimer, S.R.; Lawson, C.S.; Westbury, D.B.; Harris, S.J.; Woodcock, B.A.; Brown, V.K. Hay strewing, brush harvesting of seed and soil disturbance as tools for the enhancement of botanical diversity in grasslands. *Biol. Conserv.* **2007**, *134*, 372–382. [\[CrossRef\]](#)
23. Grime, J.P. *Plant Strategies, Vegetation Processes, and Ecosystem Properties*, 2nd ed.; Wiley: Chichester, West sussex, UK, 2001.
24. Čop, J.; Vidrih, M.; Hacin, J. Influence of cutting regime and fertilizer application on the botanical composition, yield and nutritive value of herbage of wet grasslands in Central Europe. *Grass Forage Sci.* **2009**, *64*, 454–465. [\[CrossRef\]](#)
25. Chytrý, M.; Hejčman, M.; Hennekens, S.M.; Schellberg, J. Changes in vegetation types and Ellenberg indicator values after 65 years of fertilizer application in the Rengen Grassland Experiment, Germany. *Appl. Veg. Sci.* **2009**, *12*, 167–176. [\[CrossRef\]](#)
26. Poschlod, P.; Bonn, S. Changing dispersal processes in the central European landscape since the last ice age: An explanation for the actual decrease of plant species richness in different habitats? *Acta Bot. Neerl.* **1998**, *47*, 27–44.
27. Bruun, H.H.; Fritzboeger, B. The Past Impact of Livestock Husbandry on Dispersal of Plant Seeds in the Landscape of Denmark. *AMBIO A J. Hum. Environ.* **2002**, *31*, 425–431, 427. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Hyde, B.; Carton, O.T. Manure management facilities on farms and their relevance to efficient nutrient use. *Proc. Fertil. Assoc. Irel.* **2005**, *42*, 27–43.
29. Kirk, J. The Survival and Transport of Seeds of Wild Oat (*Avena fatua* L.) in Agricultural Situations. Ph.D. Thesis, Queen's University Belfast Belfast, Belfast, Northern Ireland, 1975.
30. Humphreys, J.; Culleton, N.; Jansen, T.; O'Riordan, E.; Storey, T. Aspects of the role of cattle slurry in dispersal and seedling establishment of *Rumex obtusifolius* seed in grassland. *Ir. J. Agric. Food Res.* **1997**, *36*, 39–49.
31. Nesheim, L. Slurry seeding in grassland in Norway. In *Grassland in a Changing World, Proceedings of the 23th General Meeting of the European Grassland Federation, Kiel, Germany, 29 August–2 September 2010*; Mecke Druck und Verlag: Duderstadt, Germany, 2010; pp. 181–183.
32. Volden, B. Renewal of grassland without ploughing by wet sowing techniques, with special attention to establishment of clover in the plant stand. In *Abstracts of the 5th Circumpolar Agricultural Conference, Umeå, Sweden, 27–29 September 2004*; Swedish University of Agricultural Science: Uppsala, Sweden, 2004; pp. 37–38.
33. Jones, E.L.; Roberts, J.E. Sward maintenance of *Lolium multiflorum* by slurry seeding. *Grass Forage Sci.* **1989**, *44*, 27–30. [\[CrossRef\]](#)
34. Kader, M. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *J. Proceeding R. Soc. New South Wales* **2005**, *138*, 65–75. [\[CrossRef\]](#)
35. Šarapatka, B.; Holub, M.; Lhotská, M. The Effect of Farmyard Manure Anaerobic Treatment on Weed Seed Viability. *Biol. Agric. Hortic.* **1993**, *10*, 1–8. [\[CrossRef\]](#)
36. Weed Seed Survival in Anaerobic Digesters. Available online: <https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=1c10f5599ebba60ca9e7560ff8b389a1266c9f97> (accessed on 31 August 2023).
37. Zaller, J.G. Seed germination of the weed *Rumex obtusifolius* after on-farm conventional, biodynamic and vermicomposting of cattle manure. *Ann. Appl. Biol.* **2007**, *151*, 245–249. [\[CrossRef\]](#)
38. Wollan, E.; Davis, R.D.; Jenner, S. Effects of sewage sludge on seed germination. *Environ. Pollut. (1970)* **1978**, *17*, 195–205. [\[CrossRef\]](#)
39. Wong, M.H.; Cheung, Y.H.; Cheung, C.L. The effects of ammonia and ethylene oxide in animal manure and sewage sludge on the seed germination and root elongation of *Brassica parachinensis*. *Environ. Pollut. Ser. A Ecol. Biol.* **1983**, *30*, 109–123. [\[CrossRef\]](#)
40. Harrigan, T.M.; Mutch, D.R.; Snapp, S.S. Manure Slurry-Enriched Micro-Site Seeding of Biosuppressive Covers. *Appl. Eng. Agric.* **2006**, *22*, 827–834. [\[CrossRef\]](#)
41. Allred, S.E.; Ohlrogge, A.J. Principles of Nutrient Uptake From Fertilizer Bands. VI. Germination and Emergence of Corn as Affected by Ammonia and Ammonium Phosphate1. *Agron. J.* **1964**, *56*, 309–313. [\[CrossRef\]](#)
42. Suarez, E.A. Slurry Seeding Cover Crops. Master's Thesis, Purdue University, West Lafayette, IN, USA, 2013.
43. Hoekstra, N.J.; Bosker, T.; Lantinga, E.A. Effects of cattle dung from farms with different feeding strategies on germination and initial root growth of cress (*Lepidium sativum* L.). *Agric. Ecosyst. Environ.* **2002**, *93*, 189–196. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.