diversity

MDPI

Article

Risk of Invasive *Lupinus polyphyllus* Seed Survival in Biomass Treatment Processes

Marjaana Hassani [1,*], Elisa Vallius [1], Saija Rasi [2] and Kai Sormunen [3]

[1] Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland; elisa.vallius@jyu.fi
[2] Natural Resources Institute Finland, Survontie 9, 40500 Jyväskylä, Finland; saija.rasi@luke.fi
[3] Mustankorkea Ltd., Kivääritehtaankatu 6 C, 40100 Jyväskylä, Finland; kai.sormunen@mustankorkea.fi
* Correspondence: marjaana.p.hassani@jyu.fi

Abstract: Invasive plant species threaten native species and habitats causing ecologic, economic and social burden. When creating climate friendly solutions by utilizing plant biomasses in biogas and fertilizer production, safety should be ensured concerning the use of residues. This study concentrates on the treatment of biomasses containing invasive plant material by tunnel and windrow composting, and by farm-scale and laboratory-scale anaerobic digestion (AD) in mesophilic conditions. Germination of the nationally settled and harmful invasive species *Lupinus polyphyllus* Lindl. was investigated after these processes. In addition, the role of the conditions found in the processes that destroyed seeds were studied, such as the time of exposure, temperature and static pressure. Dormant seeds are well protected against harsh conditions and can survive through various stress factors, but also become vulnerable as more factors are combined and time of exposure is extended. Our results suggest that the risks involved for the utilization of harmful invasive species increase with mesophilic temperatures and single treatments if the processing conditions are not stabile. One-month treatment with windrow composting showed a high risk for dormant seeds of *L. polyphyllus* seeds to survive, whereby extending the processing time reduced it substantially. Hard coated seeds can thus be broken with a combination of thermophilic temperatures, moisture and static pressure.

Keywords: invasive alien species; mesophilic anaerobic digestion; seed germination; static pressure; temperature; tunnel composting; windrow composting

Citation: Hassani, M.; Vallius, E.; Rasi, S.; Sormunen, K. Risk of Invasive *Lupinus polyphyllus* Seed Survival in Biomass Treatment Processes. *Diversity* **2021**, *13*, 264. https://doi.org/10.3390/d13060264

Academic Editors: Michael Wink and Sven Jelaska

Received: 24 May 2021
Accepted: 9 June 2021
Published: 11 June 2021

1. Introduction

Invasive alien species (later IAS) cause a huge threat to global biodiversity [1] because of their impact on structure and the functioning of ecosystems [2]. IAS may cause vegetation changes and even threats to currently common native species [3,4]. Strongly growing populations may affect the number of native plant species and their allocation to reproduction [5]. Many pressures of biodiversity also result from synthetic fertilizer production and their use [6,7], and the use of land for growing energy crops [8,9]. To decrease the need of synthetic fertilizers and to increase the production of renewable energy, mowed road verge vegetation, garden waste and other unintentionally grown plant biomass could be used to produce biogas and nutrient-rich sludge. Collecting mowed plant biomass for biogas production could benefit both energy production and the status of the natural environment as carbon and nutrients are removed from eutrophicated sites [10,11]. The removal of plant biomass after mowing supports meadow plant biodiversity [12,13]. Biomass removal could improve the effectiveness of control of invasive species [14–16] and help to preserve original meadow vegetation [17]. Invasive plant biomass may also be used as a main material for bioenergy production [18,19]. Both the sludge from the anaerobic digestion (AD) process and composted plant material could then be used in soil improvement [20]. Products of composting or AD (digestate) are usually recycled as

compost mold or as fertilizers [20,21]. Digestate may be later composted but residues can also be utilized directly [22].

Composting of organic masses can be done in a tunnel or windrow compost, and the process has multiple stages [23]. Efficient composting is a result of a complicated process dependent on many variables such as time, temperature, oxygen availability, microbe community and moisture [24]. These factors are controlled by mixing different kinds of organic masses together, and with turn-arounds that maintain adequate oxygen levels. After initiation, as the process develops, the temperatures range from thermophilic to mesophilic [25]. Additionally, the pH may vary from acidic (4.5) at the beginning of the process up to 8–9, and the interaction pH and temperature may considerably affect the microbial activity [25]. Biomasses can be handled inside tunnels with moisturizing or outside in ricks where moisturizing is taken of care naturally [23].

Organic waste fractions are commonly processed in wet AD, where the total solids (TS) content is <11%. Organic materials with high TS (>15%) content such as agricultural wastes can be treated in a dry AD process but the process is less known compared to wet AD [26]. Dry AD can be done in a patch reactor or in one-, two- or multi-stage continuously fed systems, the main factors affecting anaerobic digestion being time, pH, temperature, microbe activity and moisture [27]. Unlike in composting, in dry AD, the temperature is kept constant and the moisture is controlled by watering or with percolate recirculation [26]. Most reactors operate either at mesophilic (optima at 35 °C) or thermophilic (optima at 55 °C) temperatures [27]. The optimal pH is 6.8–7.2 [27] but the process may tolerate variation from 6.5 up to 8.0 [28].

Plant biomass that can be used as main or additional material in composting and AD is usually strongly impacted by human activities. The species found on road verges and wastelands often originate from agriculture (e.g., hay species and weeds) or private gardens [29]. Some of these are strongly dispersing alien species, which dominate the vegetation in many areas [30]. If the biomass used in compost or AD contains weed seeds [31] or plant parts of invasive alien species, their use can cause a risk of spreading harmful species if all parts are not destroyed in the process. The greatest risk comes from seeds that are adapted to survive in varying conditions and germinate only when conditions for growth are favorable. Seeds can stay dormant for long period in the ground, and different species have different requirements for the dormancy to be broken [32]. Knowledge on the mechanisms of dormancy is scarce [33] but it has been shown that hard-coated and water-impermeable seeds have the best durability against harmful conditions [31,34]. Species responses to treatments may vary with different factors being crucial, as previous studies have shown [31,32,34], and complete understanding is still missing. Ensiling before AD can reduce seed survival [31] but is not always executed before processes. The high temperatures reached in the composting process have been shown to be very effective in destroying pathogens [35], and solarization is used to kill weed seed on agricultural land [34], but knowledge on preventing invasive plant propagation from composted material is limited [36]. Blumenthal et al. [37] found that using composted manure and other biosolids enhanced the invasion of *Bromus tectorum* in semi-arid rangeland, and risks related to using composted and AD processed material have also been found with the highly harmful *Eichhornia crassipes* [38].

In Finland, road verges are regularly mown but the plant material is not usually removed after mowing. However, manure and plant material from low-production lands and private gardens are used to produce biogas and garden mold products. To enhance the production of bioenergy and nutrient recycling, the use of unintentionally grown plant material should increase. However, the risk of spreading weed or invasive plant species to new areas must be taken seriously. For example, the safe use of plant biomass, including invasive plants commonly found in many areas, needs thorough investigation of the risks caused by durable seed material.

Large-leaved or garden lupin (*Lupinus polyphyllus*, Watson 1873) is a common species in Finland. It has been classified as a nationally harmful invasive plant species, and

a strategy for invasive species [39] with a management plan [40] is setting the guidelines for controlling it. The species has been found to decrease both lepidoptera and plant diversity [14], and it is considered to cause a threat to, e.g., endangered *Campanula cervicaria* and other meadow species [39] by acting as an environment engineer. This perennial herb, native to North America, is invasive in Europe, Australia, New Zealand and Chile [41]. In Finland, aggressive spreading can be seen especially on road verges and wastelands, but genetic analyses have supported the idea of separate invasions from multiple sources instead of one or a few sources [42]. Dispersal happens mainly abiotically via water, garden waste, land moves or by vehicle tires as well as with excavation activities [40,41]. Garden lupin can sometimes flower even in first summer [16] but more commonly in the second year [43]. The lifespan of garden lupin is around 20 years [44]. The species is very modest with habitat requirements and produces a large amount of durable hard-coated [45] and small (average 4 mm length) seeds. The coloration of seeds is highly variable [46], and the coloration is connected to viability and timing of germination [47]. One inflorescence produces usually a few hundred seeds but can reach up to thousand seeds per plant [44–46]. Seeds mature throughout summer and stay in dormant stage throughout winter. The longevity of *L. polyphyllus* seed has been shown to be short-term in topsoil but if seeds are buried deeper, persistence is probably several years [48], and has been estimated to be over 50 years in controlled seed storage [49]. Garden lupin has the ability to utilize a whole growing season, as it is one of the first species to emerge and can reproduce until the end of growing season. It also has a remarkable compensating capacity, from personal observation in [16].

The aim of this study was to search for the risks involved if unintentionally grown plant biomass containing IAS plants and plant parts is used in biogas or compost material production. The germination potential of common, nationally invasive and highly harmful garden lupin (Large-leaved lupin) *Lupinus polyphyllus* seed was studied to see if seed viability was affected in municipal tunnel or windrow composting processes or in farm-scale or laboratory-scale AD process in mesophilic conditions. Seeds of *L. polyphyllus* are capable of after-ripening and germinating even if vegetation is mowed in the early phase of seed ripening [47]. Germination potential increases with time, and therefore desiccated and hard seeds may prove to be resistant even in extreme conditions. Commercial seed with American origin Russel lupin (*L. polyphyllus* x *Regalis*) and natural seed from Finnish populations (*L. polyphyllus* Lindley) were used to compare their longevity in these extreme conditions.

2. Materials and Methods

2.1. Seed Material and Preparation

Seeds from garden lupin (Washington lupin) and Russel lupin were used in the experiments as there may be differences in reproductive traits between naturalized and introduced plants [50]. The naturalized species *Lupinus polyphyllus* Lindley (garden lupin) is a garden escapee and commonly found in Finland [46]. Russel lupin *Lupinus* × *regalis* (*Lupinus arboreus* × *polyphyllus*) was hybridized in England in early 19th century, resulting in an ornamental plant with branched stems and yellow flowers (in addition to white and purple found in *L. polyphyllus*) inherited from *L. regalis* [41,51]. Both taxa reproduce sexually, hybridizing with other lupin species and are perennial [45].

Seeds of *L. polyphyllus* Lindley used in experiments were hand-collected by Villi Vyöhyke ry from several areas in Tampere, Finland, in 2017. Fully matured and cleaned seeds were stored in paper bags in room temperature (±20 °C). Russel lupin seeds were ordered from Everwilde Farms USA in early 2018. In most cases, one experimental set contained 100 randomly chosen seeds. Seeds were packed in polyester bags, each of which contained 20–50 seeds either from garden lupin or Russel lupin. These small bags were then packed into larger polyester bags or plastic capsules with holes to keep them together and detectable in large biomass. Larger polyester bags exposed seed samples to more direct contact with biomass and static pressure compared to plastic capsules.

2.2. Composting Experiments

The composting experiments included 29-day tunnel composting, 30-day windrow composting and 119-day windrow composting, in which different raw material mixes were used (Table 1).

Table 1. Details of composting experiments. Lindl. refers to L. p. Lindley and Reg. to Russel lupin.

Code	Experiment	Material	Duration	Timing	N of Seeds
T1	Tunnel 1	Source separated and crushed biowaste, ash, woodchips	29 days	Winter 2018	Lindl. = 965 Reg. = 300
T2	Tunnel 2	Sewage sludge, ash, woodchips	29 days	Winter 2018	Lindl. = 1078 Reg. = 300
A11	Windrow rick 1	Garden waste (70%), crushed and pre-treated biowaste (30%)	30 days	Winter 2018	Lindl. = 280
A12	Windrow rick 2	Garden waste (70%), woodchips (15%), crushed and pre-treated biowaste (15%)	30 days	Winter 2018	Lindl. = 300
A2	Windrow 2	Garden waste (70%), woodchips (15%), crushed and pre-treated biowaste (15%)	119 days	Summer 2019	Lindl. = 800 Reg. = 660

In the tunnel experiments (T1 and T2), the starting pH was 8 and the average VS was 57.8%. Compost mass was not irrigated in the tunnels. In windrow experiments, starting pH was 6.6–7.2 and the average VS was 64.25–72.9% (finishing pH and VS: 7.7–9.1 and 65.8–75.1%, respectively). Ricks were set outside under natural rainfall. Polyester bags and/or capsules contained seed samples that were set into three different layers: bottom, middle and top (Figure 1). In tunnel experiments (T1 and T2), the bottom layer was approximately at 3.4 m depth from the surface, the middle layer was at around 1.5 m depth from the surface, and the top layer was on top of the biomasses, whereas in windrow composts (A11, A12 and A2), the top samples were placed at 0.5 m depth from surface. In all composting experiments, mass was regularly turned to raise oxygen levels maintaining microbial activity and keeping the temperature high, as well as to mix biomasses to be treated evenly. After turn-arounds samples were set back into same layer but exact location might change inside a layer. Turn-around was done once per week in the tunnel and one-month windrow compost experiments, and once per month in the four-month windrow composting (A2). In the four-month experiment some samples were also moved among the layers. Some samples were removed during turn-arounds for a shorter treatment time. After the experiments, seed samples were washed with tap water and set into germination tests.

2.3. Anaerobic Digestion (AD) Experiments

To be able to compare the different biomass treatment processes, farm-scale AD and laboratory-scale biochemical methane potential (BMP) experiments were conducted. The farm-scale AD experiments took place in winter 2018–2019 (M1, 155 days) and spring–summer 2019 (M2, 132 days) in Finland. In farm-scale AD patch-reactors, the raw material consisted of residual hay from agriculture. pH during the process was on average 7–8. The density of biomass was 800–900 kg m^{-3} and the pile of hay was 3 m high. Static pressure inside mesophilic patch-reactor is 0.1 bar per one meter of biomass. In the patch-reactor, biomasses are moisturized from top and percolation liquid is circulated, and temperature is kept in 37 °C. The in patch-reactor biomass was set inside the reactor (Figure 2) and the biomasses was not mixed during the process. The seed samples were set into three different layers: bottom, middle and top. The bottom layer was in approximately 3 m depth from surface, the middle layer was around 1.5 m depth from surface, and the top layer was on top of the biomasses. The control sample was kept inside reactor, but not in contact with the biomasses. Four part-samples were set into the bottom and middle layers with 25 seeds inside, and two part-samples were set into the top layer with 50 seeds inside.

Only garden lupin (*L. polyphyllus* Lindl.) seeds were used in the experiment. The samples were collected after treatment, washed with tap water and set into the germination test.

Figure 1. Four-month windrow composting experiment at the facilities of Mustankorkea Ltd. Seed samples were set inside small polyester bags and either a bigger polyester bag or a plastic capsule as part-samples. Temperature loggers were set inside the capsules and the samples were in three different layers. (Photo: Marjaana Hassani).

Figure 2. The farm-scale patch-reactor samples were set inside polyester bags that were set into the bottom, middle and on top of the biomasses. (Photo: Marjaana Hassani).

The seed viability was also tested after BMP assays in mesophilic (37 °C) conditions in spring 2021 at the University of Jyväskylä. BMP assays were conducted in 1L bottles with 700 mL liquid volume. Separately collected biowaste was used as substrate and the inoculum used originated from Mustankorkea biogas plant (treating mainly source separated municipal biowaste). A ratio of volatile solids (VS) of the substrate and inoculum ($VS_{substrate}/VS_{inoculum}$) of 0.5 was used. $NaHCO_3$ (3g/L) was used as the buffer and distilled water was added to achieve 700 mL volume. Half of the seeds were left intact

(A samples) but half (B samples) were scarified by 1 min shaking in a glass jar. A total of 25–100 seeds were placed in each bottle but layer-effect was excluded. All bottles were flushed with N_2 to obtain anaerobic conditions. Bottles 1A-1B were set for 10 days and 2A-5B were set for 30 days and mixed with shaking daily. Methane production of the inoculum only was subtracted from the results containing both the substrate and inoculum to achieve methane production of the feedstock. pH was measured in the beginning and after the experiment using VWR pH100. Gas production and concentration were measured with Optima 7 Biogas Analyzer several times during experiment. The control sample without treatments was used for a viability check. After 10 and 30 days of experiment, seed samples were washed with tap water several times and seeds were taken into the germination test.

2.4. Measurements

The environmental factors found in the experimental process and affecting seed viability were treatment time, temperature, pH and static pressure. Moisture was not observed as it could not be efficiently controlled and measured. Phytotoxicity and microbial activity was also left out from review. Seed samples were classified into time groups (Time_c) based on time spent in treatment; control samples that did not include time variables were in group 0, 7–17 days in group 1, 29–30 days in group 2, 119–132 days in group 3 and 155 days in group 4.

Temperature variations were followed in composting processes by using temperature loggers (Thermochron DS1921G) that measured temperature once per hour. In the tunnel and first windrow composting experiment, loggers were set inside capsules: two loggers into the bottom, two into the middle and one into the top layer. In the second windrow composting experiment, loggers were set inside capsules: two loggers into the bottom, one into the middle and one into the top. Since the capsules were able to move within the layer in turn-arounds, the measurements varied depending on each position inside the rick.

Temperature measurements were evaluated from each experiment. Both the one-month windrow and the tunnel composting experiments had all the data measured, but from the four-month windrow composting the data was lost and provided only for the last one-month period. The temperature data was divided into sections between turn-arounds, since the temperature dropped down while the samples were removed from the compost and increased again as the samples were returned into the compost. Temperatures that referred to inside or outside temperatures were removed from the data. The data was separated by layers and the temperature variation was evaluated between each layer´s logger. From each composting experiment, the number of days when the mean temperature did not exceed 30 °C (Days_30) and days when the mean temperature was 50 °C or more (Days_50) were calculated. The measured temperature data was used to classify the layers into mesophilic (1) and thermophilic (2) groups, and the control samples that were not temperature dependent (0) in their own group (Temp_c).

Static pressure affecting the seed samples was estimated based on the biomass density measurements and maximum height of the ricks. As the exact pressure could not be measured, the static pressure affecting the seed sets were based on the depth of the layer in which they were placed. Because the static pressure on seeds packed with or without the capsule were probably slightly different, rough estimates were used.

2.5. Seed Viability

The seed viability was tested with the traditional germination test. After treatments, seeds that were visibly recognized as destroyed were removed. Destroyed seeds were either rotten and softened or they had completely lost structure. The remaining seeds were set between moisturized filter paper that was then rolled into open plastic bottles. The bottles were stored in a growth-chamber that was set into suitable germination conditions of 17 °C. The light in the growth-chamber followed a day–night cycle of 16–8 h. The germinated seeds were removed during the check-ups and the papers were moisturized

during checking, but the filter papers were able to dry between check-ups. Filter papers were replaced irregularly based on their condition. Seeds that were able to grow primary roots (or stem and leaves), were considered as viable. Seeds that stayed in the dormant stage were considered as at high-risk of germination. Some seeds started to decay during germination tests and were classified as destroyed. The condition of seeds was evaluated throughout the germination test. Germination was followed for 1–2.5 years for composting and farm-scale AD experiments, and about three months for BMP experiment. The differences in the following of the germination times was due to different ending times of the experiments as the experiments were conducted in 2018–2021. The proportion of different viability groups (viable_%, dormant_%, destroyed_%) was calculated. The viability of the control samples left outside of the experimental process was tested accordingly after breaking dormancy using scarring or short hot-water treatment required to induce germination of the non-treated seeds.

2.6. Statistical Testing

To explore the effect of different in-process variables on seed survival, a Poisson log-link generalized linear mixed effect model (GLMM) was used. Separate models were used for composting, farm-scale AD and BMP experiments, as the datasets were unbalanced due to differences in the number of replicates. For the composting experiments a generalized linear model was construed with a response variable of log (viable_%) determined with categorical explanatory variables of treatment (time_c, temp_c, layer and interaction of time_c and layer, temp_c and layer) and days _30 and days_50 were included as covariates. The model was tested with destroyed_% and dormant_% and the model with the lowest AIC value was chosen. For AD a Poisson log-linear GLMM with viable_%, dormant_% or destroyed_% as dependent variable was determined with categorical explanatory variables time_c and layer and their interaction. The Wald chi square test was used to test statistically significant differences. The effect of the seed material was compared in composting processes (T1, T2, A2) and in the BMP experiment, in which the seeds with Russel and garden lupin were placed in the same bags/capsules/bottles. A non-parametric Wilcoxon rank sum test was used to test the statistically significant differences among the origins. All statistical tests were conducted using IBM SPSS Statistics (version 26).

3. Results

3.1. Composting Experiments

The results from different composting experiments show that multiple factors have effect on the seed viability of the garden lupin (*L. polyphyllus* Lindley) (Table 2). The seeds were more likely to be viable if the time of treatment was shorter (30 days) and the temperature mesophilic ($p < 0.001$). There was no significant difference in the survival between seeds placed in bags or capsules. Seed viability was affected by time, temperature, layer and their interactions, and covariates (days_ 30 and days_50) especially related to proportion of viable seeds ($\chi^2 = 555.927$, df = 10, $p < 0.001$). Seeds were less likely to survive in the top (−24.747 b) and the middle (−24.989 b) layers than in the bottom of the compost (Figure 3). If interaction with temperature and layer was included, it also appeared significant ($p < 0.001$). Especially mesophilic temperature in the top or middle layers had positive effect on the seed viability, meaning higher risk for seed survival through the process. The proportion of seeds in dormancy after the process was affected by time, layer, days_50 and interaction of temperature and layer ($\chi^2 = 2003.771$, df = 10, $p < 0.001$). Seeds were more likely to be dormant in 30 days than in 119 days compost (2.644 b), and less likely to survive dormant from the top (−24.689 b) and middle layers (−24.487 b). The proportion of destroyed seed was connected to days in <30 °C, time spent in treatment and their interaction ($\chi^2 = 323.059$, df = 13, $p < 0.001$).

Table 2. Results from generalized linear mixed models used to determine the effects time, temperature and layer on the proportion of destroyed, dormant and viable *L. polyphyllus* Lindl. seed after composting processes.

Response Variable	Explanatory Variable	χ^2	df	p [1]	N
Viable_% (Lindley)	(Intercept)	119.795	1	***	73
	Time_c	28.450	1	***	
	Temp_c	8.369	1	**	
	Layer	60.656	2	***	
	Days_30	9.074	1	**	
	Days_50	63.599	1	***	
	Temp_c * Layer	15.579	2	***	
Dormant_% (Lindley)	(Intercept)	496.138	1	***	73
	Time_c	52.011	1	***	
	Temp_c	4.525	1	*	
	Layer	27.969	2	***	
	Days_30	3.673	1	0.055	
	Days_50	88.871	1	***	
	Temp_c * Layer	57.34	2	***	
Destroyed_% (Lindley)	(Intercept)	352.026	1	***	86
	Time_c	169.887	2	***	
	Temp_c	7.774	1	**	
	Layer	3.8	2	0.15	
	Days_30	18.65	1	***	
	Days_50	2.63	1	0.105	
	Time_c * Layer	48.288	4	***	
	Temp_c * Layer	25.531	2	***	

[1] * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

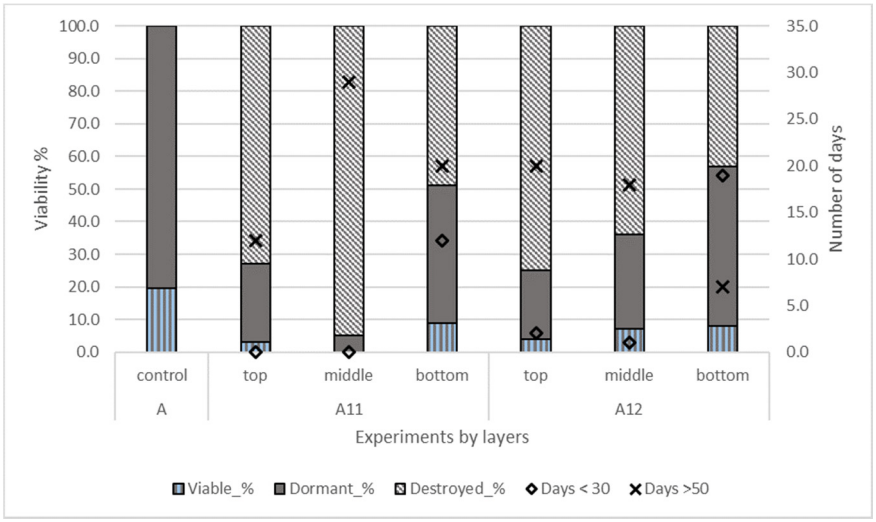

Figure 3. Proportion of viable, dormant and destroyed *L. polyphyllus* Lind. seed after windrow (A11, A12) composting and control shown separately for compost layers (top, middle and bottom). Number of days < 30 °C and days > 50 °C are presented.

As the results of the composting experiments were divided by process time, the significance of temperature was revealed (Figure 3). Seeds were less likely to survive if number of days with >50 °C rise and number of days with <30 °C stayed low. One-month of windrow composting has risk for seed survival (viable seeds on average 5.1% and dormant seeds on average 28.4%, N = 580). A larger proportion of seed survived either as viable or dormant in mesophilic temperatures compared to thermophilic stage (Figure 4). However, temperatures varied between layers largely depending on the outer and inner parts of the ricks (Figure 5). In the 30-day windrow composting experiments (A11, A12),

days in which mean temperature stayed under 30 °C were mostly in the bottom layers (12 and 19 days in A11 and A12, respectively). Both ricks also had several days in which temperature exceeded 50 °C (29 days in middle layer of A11 and 20 days in top layer of A12). In one-month windrow experiments A11 (N = 280) and A12 (N = 300) only capsules were used excluding the effect of static pressure. The temperature mode in the top layer was between 49.5–62 °C (SD 5.6–10.7), in the middle layer—42–71 °C (SD 7.4–12.5) and in the bottom layer—23.5–54.5 °C (SD 5.9–11.6).

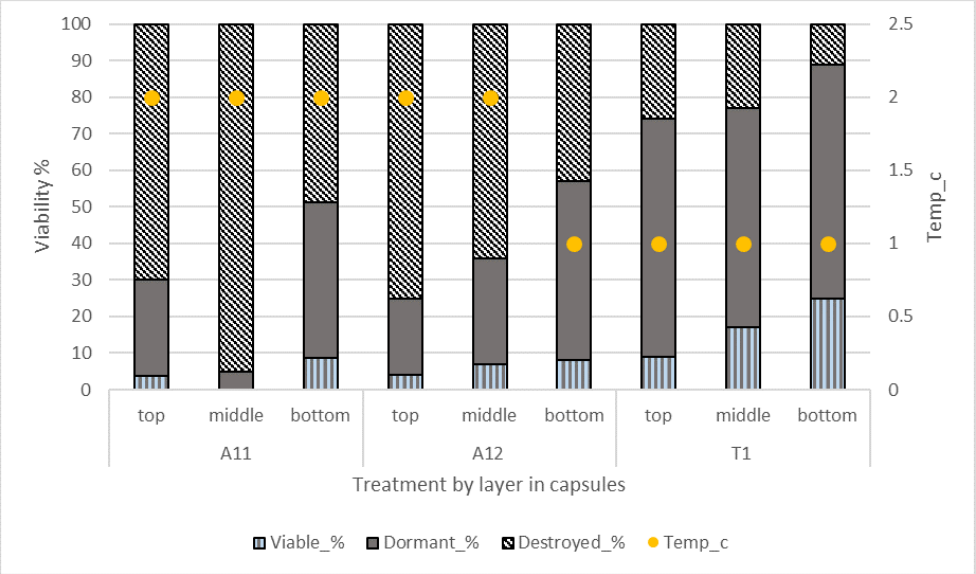

Figure 4. Proportion of viable, dormant and destroyed *L. polyphyllus* Lind. seed after windrow (A11, A12) and tunnel (T1) composting (bars) showing average temperature classified as mesophilic (1) and thermophilic (2).

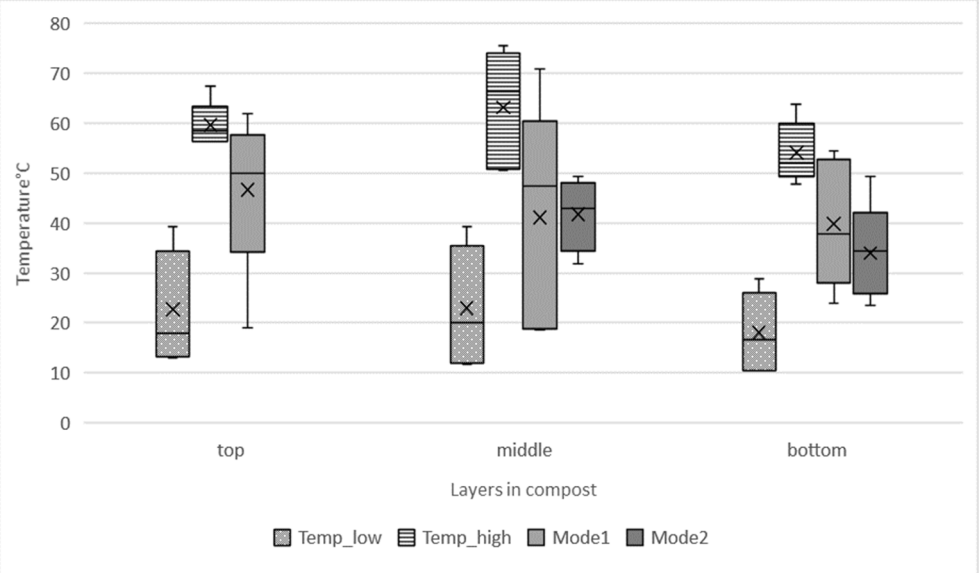

Figure 5. Temperature variation between layers (top, middle and bottom) in windrow (A11, A12, A2) and tunnel (T1, T2) composting experiments. Each layers loggers lowest and highest temperatures (Temp_low, temp_high) were measured as well as mode (mode 1–2) variations between treatments.

Results from the one-month tunnel composting experiments (T1, N = 965 and T2, N = 1078) were compared to the four-month windrow composting (A2, N = 800) with higher temperatures. Higher temperature and longer treatment time (119 days) lowers survival

rate significantly in the windrow composting experiment compared to the 29-day tunnel composting. However, some seeds were able to survive even through the four-month windrow composting (Figure 6) even though the proportion of viable seed decreased from 16.7% to 0.1% and that of the dormant seed from 61.6% to 0.6%. All differences cannot be explained by temperature, as in T2, samples were packed inside polyester bags as in other treatments, capsules excluding static pressure and decreasing the contact with biomass were used. As static pressure (approximately top 324.5 kg/m^2, middle 973.5 kg/m^2 and bottom 2206.6 kg/m^2) was included in samples of T2, even lower temperatures in bottom layer did not raise the proportion of viable (14%) or dormant (49%) seeds. Control samples used in the tunnel experiments had remarkably high proportion of viable seed and control in windrow experiment (A2), the proportion of dormant seed was over 80% (Figure 6A).

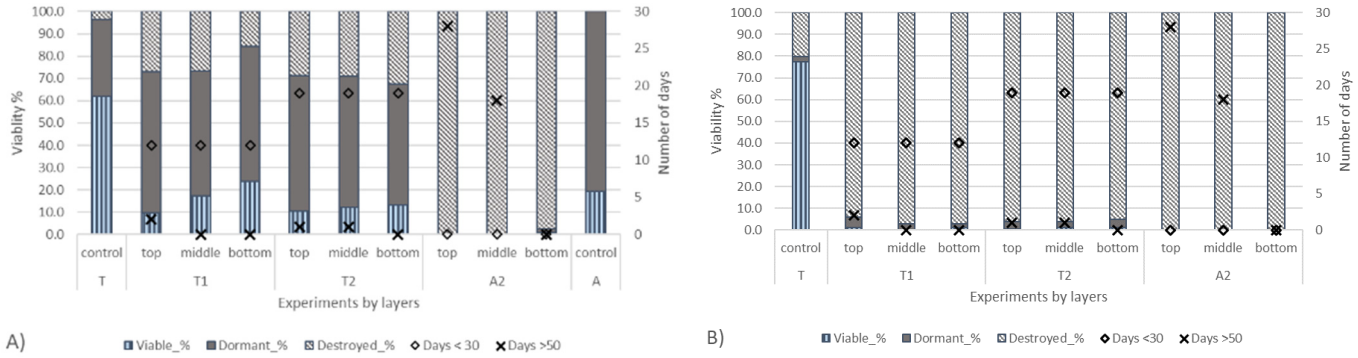

Figure 6. Proportion of viable, dormant and destroyed (**A**) *Lupinus polyphyllus* Lindley and (**B**) *L. polyphyllus* × *Regalis* after one-month tunnel (T1, T2) and four-month windrow composting experiment (A2) and in their control samples. Results are separated based on layers (top, middle and bottom). Temperature is included as days < 30 °C and days > 50 °C for each layer during one month.

In a one-month tunnel composting experiment, a greater proportion of seeds with Russel lupin were destroyed compared to Finnish garden lupin (Wilcoxon = −3.417, N = 15, p = 0.001 and W = 2.668, N = 9, p = 0.008, for T1 and T2, respectively, Figure 6B). From the one-month tunnel experiments only 0.7–0.9% of Russel lupin seeds were viable (SD = 1.8–1.9), 3.3–3.4% were dormant (SD 3.1–5.0) and 95.8–96% were destroyed (SD 3.9–6.75). In the four-month windrow compost, all seeds from Russell lupin were destroyed (Figure 6B) and there was no significant difference between seeds with different origins (W = −1.837, N = 28, p = 0.102). In the control samples, 77.3% from the Russell lupin seed were classified as viable, 2.7% dormant and 20,1% destroyed.

3.2. Anaerobic Digestion in Mesophilic Conditions

Results from the farm-scale patch-reactor with garden lupin seeds revealed that the time in process, layer, and their interaction (χ^2 = 24.953, df = 5, $p < 0.001$) affected the proportion of viable seeds (N = 20, SD = 1.821, Table 3). Shorter treatment time (27.614 b) increased the proportion of viable seeds, whereas location in the middle layers decreases it. The probability of dormant seeds increases in the top layer ($p < 0.001$). Less dormant seeds were found from the middle layer than from the bottom, and seeds were more likely to be dormant in the shorter treatment time and in the top layer (χ^2 = 355.740, df = 5, $p < 0.001$). The seeds were most likely destroyed (χ^2 = 82.557, df = 6, $p < 0.001$) in the middle layers.

Table 3. Results from generalized linear mixed models used to determine the effects time, temperature and layer in the biomass on the proportion of destroyed, dormant and viable *L. polyphyllus* Lindl. seed after farm-scale AD process.

Response Variable	Explanatory Variable	χ^2	df	p [1]	N
Destroyed_% (Lindley)	(Intercept)	15229.79	1	***	21
	Time_c	17.871	1	***	
	Layer	42.852	3	***	
	Time_c * Layer	27.155	2	***	
Dormant_% (Lindley)	(Intercept)	133.664	1	***	20
	Time_c	0.789	1	0.374	
	Layer	142.91	2	***	
	Time_c * Layer	a			
Viable_%(Lindley)	(Intercept)	0.769	1	0.381	20
	Time_c	a			
	Layer	0.769	2	0.381	
	Time_c * Layer	a			

a = unable to compute. [1,*] $p < 0.05$; *** $p < 0.001$.

In the farm-scale AD in the mesophilic patch-reactor the treatment time is long (155 and 132 days for M1 and M2, respectively) but because the temperature is kept in mesophilic conditions, its effect on seed destruction decreases (Figure 7). Only polyester bags were used in the AD experiments, allowing static pressure to have an impact (approximately 3N on bottom layer, 1.5N in middle layer and 0N on top). The control sample was inside the reactor without contact, but was affected by the temperature, gases and moisture, being similar to the M1 experiment's top layer. Samples that had direct contact to biomasses had lower viability, especially samples that were in the middle or bottom layers. Average viability in the AD experiments was 0.5% as viable, 6.4% as dormant and 93.1% as destroyed. The highest viability was found in the M1 experiment top layer, where 50% of the seeds were dormant (SD 8.5).

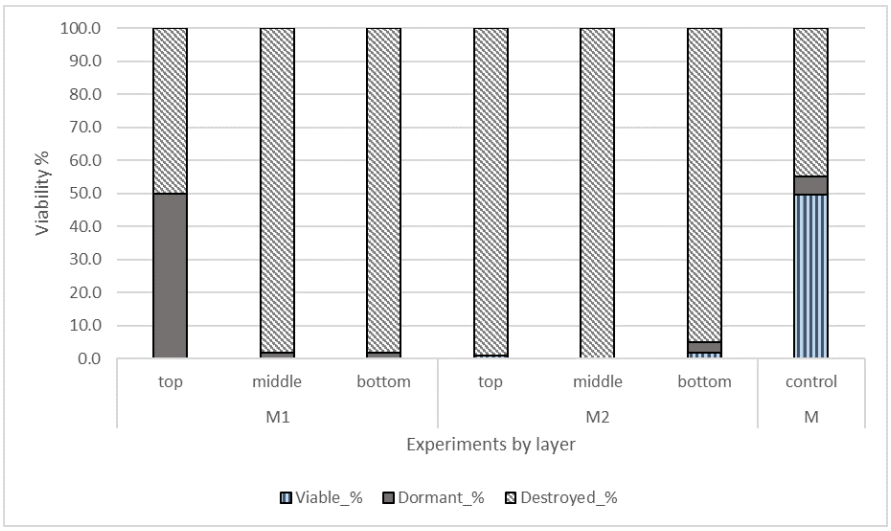

Figure 7. Proportion of viable, dormant and destroyed *L. polyphyllus* Lindl. seeds after 155 days (M1) or 132 days (M2) in farm-scale mesophilic AD process. M refers to control sample in 155 day process without contact to biomass.

The results from the laboratory BMP experiment should be considered with information from the process measurements. The pH of the digestate after 10 days was slightly lower (7.5) than after 30 days (from 7.7 to 8.1). Additionally, the methane production was about 75% lower in B1 than in the 30 days bottles (on average 345 ± 34 mLCH4/gVS, data

not shown), assumed as due to the short retention time. In A1, the methane production started slower than in the other bottles and began to decrease after day 6, indicating that the degradation process was not successful or there was a major leak in the gas bag. In the 10-day experiment (A1, B1), the viability of seeds (2–8% viable, 4–8% dormant, 88–90% destroyed) was higher than in the 30 days samples (1–1.3% viable, 0.6–4.4% dormant, 94.6–98.1% destroyed) both in garden and Russel lupin. There was a trend that more seeds of Russel lupin than garden lupin was destroyed in the BMP process (Wilcoxon: W = 1.791, N = 10, $p = 0.073$) which may be explained by the lower viability of the Russel lupin also in the control samples (Figure 8).

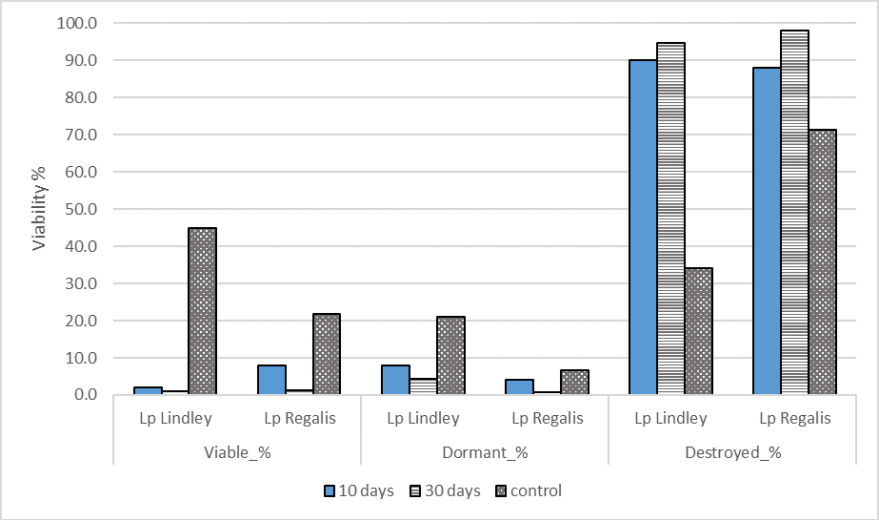

Figure 8. Proportion of viable, dormant and destroyed *L. polyphyllus* Lindl. ja *L.p.* Regalis seeds after 10 and 30-day laboratory-scale BMP experiment with control samples.

3.3. Effect of Time, Temperature and Moisture on Seed Survival

Both composting and AD experiments showed an interaction between temperature and time in relation to seed viability. In the one-month mesophilic processes, the proportion of viable seeds was 51%, the dormant was 18% higher and the destroyed was 21% lower compared to the thermophilic process (Table 4). When the mesophilic processes were compared to BMP with similar temperature and treatment time but higher moisture, the proportion of viable seed was 82%, the dormant was 91% lower and the destroyed was 91% higher in the latter (Table 4). If the treatment time was increased to 119 days, the proportion of viable seed decreased 97% and the dormant was 96%, and the proportion of destroyed seed increased by 50%. With long AD processes viability was low, but overall survival was higher than in short BMP experiment (Figure 9). Seeds had highest survival (viable or dormant) in the composting experiment of 7–17 days, 86% higher than with the 10 days BMP samples. The survival rate decreased 25% if the time was increased to 30 days. This was still 91% higher than the survival rate of the BMP experiment. The longer treatment of 132 days (AD) had only a 2.2% survival rate, but the effect of the longer treatment time is not straightforward (Figure 9). Extreme temperatures (>70 °C) with durations of 2–11 days were found in the middle layer of the one-month windrow composting experiments. Seeds placed in the same capsule with loggers measuring these temperatures were not viable but in three out of five capsules, the proportion of dormant seed was still 5–15%.

Table 4. Combined results from different experiments showing the most important findings about the effect of treatment process, duration of treatment, temperature and depth of samples (layer) on seed survival of *L. polyphyllus* Lindl. Proportions in each viability class is presented as average and total number of seeds in combined treatments.

Process	Duration/Layer	Temperature	Viable %	Dormant %	Destroyed %	N
Composting	Short (<30 days)	Mesophilic	10.4	34.6	55.0	2355
		Thermophilic	5.1	28.2	66.7	480
BMP	Short (<30 days)	Mesophilic	1.9	3.2	94.9	1220
Composting	Long (119 days)	Mesophilic	0.3	1.3	98.3	135
		Thermophilic	0	0	100	1190
AD	Long (132/155 days)	Mesophilic	0.5	6.4	93.1	600
Composting	Top ormiddle	Mesophilic	10.3	33.0	56.8	1328
AD	Top or middle	Mesophilic	0.2	9.0	90.8	400

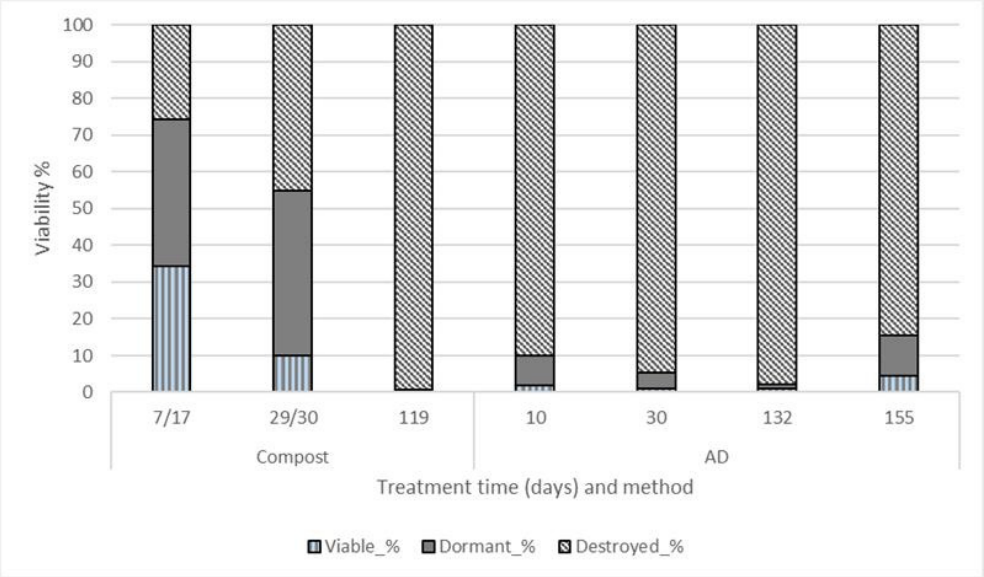

Figure 9. Proportion of viable, dormant and destroyed *L. polyphyllus* Lindl. seeds after composting and AD processes. Results from different experiments are combined based on the duration of treatment.

4. Discussion

4.1. Composting

The time in the compost process and the temperature affected the viability of the *Lupinus* seeds. In the short treatment time (30 days) and mesophilic temperature 45% of the seeds were still capable of germinating, this being relatively high if compared to other invasive species (Hassani et al. unpublished data). Seeds were less likely to survive in the top and middle layers than in the bottom of compost. This may be explained by the usually higher temperatures in the top and middle layers. Mesophilic temperature in the top or middle layers had a positive effect on seed viability, but this is more likely in cases that the composting process is not working properly and the temperature is lower than usually. The proportion of dormant seeds followed the same trend, highest risk being related to short process time and mesophilic temperatures. The method of packing seeds for the experiment (bag vs. capsule) did not affect the seed viability. This may mean that moisture content and pressure of compost mass did not have clear effect on viability, highlighting the importance of thermophilic temperatures and number of days in over 50 °C for successful destruction of harmful seed material. However, in the tunnel experiments, the results revealed that pressure had an impact on the effect of lower temperature on seed survival, which suggests that pressure might have a minor role in causing seed destruction.

In composting, treatment time is convertible, and the process can be divided to mesophilic, thermophilic, and cooling stages [52]. The temperature reached during the process depends on the material used (e.g., pH), and the oxygen levels affecting the microbial community and respiration [25]. These conditions are regulated by aeration [52] or as in this case, with turn-arounds. However, high temperatures are only found for a limited time because temperature over 45 °C inhibits the microbial activity and causes cooling of the compost mass [52]. Results from the experiments revealed that especially in windrow composting, temperatures can easily exceed 45 °C and stay surprisingly high even for longer periods. However, temperature variations may be problematic in the composting process. Experiments revealed that at the same time and experiment different parts of the same layer were able to have a temperature mode varying between 19–49.5 °C (tunnel) or 42–71 °C (windrow) (Appendix A).

The risk of garden lupin (*L. p.* Lindl.) seeds to survive from all composting experiments was on average 8.3% as viable and 31% as dormant, and with windrow composting the average was 2.6% as viable and 14.2% as dormant, whereas with Russel lupin (*L. p.* × *regalis*) it was only 0.4% as viable and 1.6% dormant. Pérez et al. [38] presented similar results (3.5 ± 0.96%) showing survival of water hyacinth (*Eichhornia crassipes*) from windrow composting of 6.5 months. If conditions of composting processes are not properly taken care of, risk for spreading viable seed material of garden lupin is high. This can be reduced with extending processing time to over 4 months and using composting methods with higher temperatures (such as windrow composting). However, a one-hour time of 70 °C is commonly used in pasteurization of compost [53] or 5–10 days sanitation in over 50 °C [54] is probably not enough to destroy all seeds, because some seeds were able to survive as dormant (0–15%), even if the temperature in the middle layer´s loggers was measured 2–11 days exceeding 70 °C. Survival of the *Lupinus* seed in dry conditions and in 70 °C was also found in controlled laboratory tests (Hassani et al. unpublished data).

4.2. AD Processes

Different types of conditions in anaerobic digestion experiments in mesophilic conditions affected seed viability more than the length of processing time alone. Especially, the effect of higher moisture can decrease *Lupinus* viability together with other factors. In shorter hydraulic retention times, *Lupinus* seeds were mostly destroyed, and only 6.3% of seeds were still capable of germinating, whereas in the longer farm-scale patch reactor 6.9% of seeds were capable of germinating. However, in the farm-scale AD experiments, a single part-samples viability in the top layer was able to rise up to 56%, highlighting the risk in single treatment. Similar risk with seed survival has been noted also in previous studies with different species [55,56]. On the contrary, fast destruction of other weed species with stirred tank reactors has also been found previously [57,58].

The type of AD process studied in this paper in the farm-scale is different than most of the AD processes in the world. This type of reactor is used when raw material is very dry and inconvenient to pump to digesters, e.g., garden or agricultural waste. The hydraulic retention time is usually much longer than in more common continuously stirred tank reactors, but the raw material is not mixed, which highlights the importance of the percolation liquid recirculation system that all raw material is fully moisturized with percolation liquid. In this study, seed material was surviving possibly due to dryer pockets or the effect of alkaline anaerobic and dry conditions that can maintain seeds in deeper dormancy (Hassani et al. unpublished data).

The usual hydraulic retention time in a continuously stirred tank reactor is from 10 to 25 days, although HRT's from 50 to 100 days are also reported depending on the raw material [59]. The 30 days experiment used in this study represents the average degradation time of municipal biowaste. The experiment indicates the importance of retention time and full degradation of the material as in the 10 days experiment the seeds were more likely to survive (10–12%). This is still a higher survival percentage than reported in earlier studies with other species [57,58] remarking the *Lupinus* high survival potential. It also should be

noted that, depending on the raw material mix of the process, commonly hygienization of material in 70 °C is required, which may have an effect also on seed survival. However, the hydraulic retention with a higher moisture content has definitely an affect on seed survival since lupin seeds are sensitive to the combination of temperature and moisture (Hassani et al., unpublished data). Previous studies also confirm that hard-coated seeds were able to survive through 30 days AD with the patch-reactor in mesophilic conditions, as water impermeability protects seeds from thermal destruction [31].

4.3. Risk of Seed Survival

When specific factors affecting the seed viability and germination of seeds are reviewed, some factors tend to arise as more important than others. Temperature is seen as one of the most important factors affecting seed viability [34,57,58] and the ability to germinate [60], and water impermeability with hard-coated seeds is important for seed survival [31,55]. The effect of temperature has been based on for example microbial activity and cell ability to survive under temperature variations, and the lower limit of hygienization is set to 70 °C. Most difficulties with seed destruction are caused by seed dormancy. *Lupinus* protects its seeds with hard seed-coat that protects it from unsuitable germination conditions (e.g., winter, heat, flooding or burial to ground), seeds mechanism with dormancy breaking are however poorly understood. Seeds can stay dormant in ground for long periods [41,61]. Dormancy can be broken e.g., by scarification, moisture and pressure. The breaking of dormancy follows natural conditions such as rapid germination under cold spring conditions [51], if the conditions are extreme the seed can stay dormant. Seeds from different treatments were reviewed several times during the germination test, and the long test time might have affected seed survival during the storage time. The extensively long germination test did also show that dormant seeds were able to germinate even after 2.5 years from the start of the test. Therefore, dormant seeds should be considered to have a high risk of germination. The extreme conditions found in the experiments may have induced deep dormancy in *Lupinus* seed.

Results with Russel lupin (*L.p.* × Regalis) showed no significant difference with time of treatment or days <30 or >50 because the seeds were mostly destroyed in all treatments. The overall risk with Russel lupin was shown to be significantly lower than with garden lupin. By adapting to a number of different habitats and environments, garden lupin has developed strong survival ability [46,62], which is shown, e.g., in seed mass and plant size [50].

5. Conclusions

Lupinus polyphyllus causes severe damage for diversity, especially by overpowering vegetation in ruderates and road verges that can be considered as surrogate areas for meadow species. Since this perennial invader reproduces mainly through massive seed production, the spread of seeds should be limited. By collecting biomasses from these areas, we could not only limit the spread of the species, but also produce renewable energy and fertilizers. Different methods of processing biomass could be used here, as they could significantly reduce the number of seeds that spread into the wild. Carefree biomass treatment could, in turn, lead to the spread of the species to new areas.

If biomasses containing harmful seed material from IAS are used in biomass treatment processes, the safest method to use would be the combination of anaerobic digestion and windrow composting. Such waste management is already in use in some public waste centers in Finland. Although the costs increase as a result of the treatments, other benefits may offset the rising costs, such as the sale of biofuels. If the moisture content can be raised, such as in the BMP experiment, an even shorter one-month treatment time would decrease the risk of IAS, especially with composting residues.

Author Contributions: M.H. conducted the investigation; formal analyses, M.H. and E.V.; writing—original draft preparation, M.H., E.V. and S.R.; writing—review and editing, M.H., E.V., S.R. and K.S.; visualization, M.H.; supervision E.V., S.R. and K.S.; financing, K.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ellen ja Artturi Nyyssösen säätiö (5/2018), Tekniikan Edistämissäätiö (4/2018), Mustankorkea Ltd. and University of Jyväskylä.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: *Lupinus polyphyllus*. Available online: https://nextcloud.jyu.fi/index.php/f/4382855 (accessed on 28 May 2021).

Acknowledgments: Open access provided by Diversity and NEOBIOTA conference. Mustankorkea Ltd. for experimental facilities and Villi Vyöhyke ry for seed material used in experiments.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

Experiment	A11	A11	A11	A11	A11		A12	A12	A12	A12	A12
Layer	Top	Middle 1	Middle2	Bottom 1	Bottom 2		Top	Middle 1	Middle2	Bottom 1	Bottom 2
Mode	51,0	61,5	69,5	62,5	58,0		44,0	68,5	63,0	29,0	27,0
Average	51,9	60,9	68,9	61,2	55,0		40,7	60,6	54,4	40,2	34,7
Min	40,5	40,5	45,5	34,0	31,5		18,0	21,0	19,0	25,0	26,0
Max	56,5	69,5	71,0	67,5	60,5		55,0	70,0	63,0	56,0	47,5
Std. dev	3,7	5,4	2,8	5,1	4,5		10,4	12,8	10,7	8,8	6,7
Mode	49,5	71,0	42,0	54,5	28,0		62,0	61,0	44,0	51,0	23,5
Average	50,2	69,7	40,3	55,7	32,8		60,2	54,3	45,1	45,8	25,1
Min	9,0	41,0	31,0	37,5	28,0		10,0	31,5	24,0	6,5	3,0
Max	54,5	74,5	52,0	64,0	53,0		65,0	65,5	49,0	57,0	32,0
Std. dev	3,5	4,4	2,9	3,3	7,2		4,9	5,8	2,3	10,1	2,3
Mode	39,0	71,0	38,0	46,0	27,0		66,0	47,5	60,5	31,0	27,0
Average	39,8	62,7	41,6	39,3	33,7		53,9	48,1	45,5	35,4	32,3
Min	29,5	32,0	32,5	26,0	11,5		25,5	31,0	15,5	18,0	1,5
Max	45,5	71,0	58,5	47,0	56,0		67,5	52,0	61,5	51,5	41,0
Std. dev	1,4	9,4	5,2	7,0	8,3		8,7	2,4	10,5	5,9	5,0
Experiment	A2	A2		A2	A2						
Layer	Top	Middle 1		Bottom 1	Bottom2						
Mode	53,5	50,0		38,0	35,0						
Average	53,4	50,0		42,5	36,2						
Min	39,5	39,5		33,0	29,0						
Max	56,5	51,0		48,0	46,0						
Std. dev	1,3	0,7		3,5	2,2						

Mesophilic 20-45 | Thermophilic 50-67 / > 55 | Hygienize 70

Experiment	T1	T1	T1	T1	T1	T2	T2	T2	T2	T2
Layer	Bottom 1	Bottom 2	Middle 1	Middle 2	Top	Bottom 1	Bottom 2	Middle 1	Middle 2	Top
Mode	32,5	34,5	18,5	32,0	19,0	24,0	49,5	19,0	49,5	50,0
Average	31,0	31,4	31,4	31,4	33,0	30,2	30,2	29,8	29,7	30,0
Min	7,0	7,5	8,5	8,0	7,0	5,5	5,0	5,5	5,5	5,0
Max	51,5	50,5	50,5	51,0	58,5	52,5	59,5	71,0	74,0	59,5
Std. dev.	9,7	9,8	10,3	10,5	11,5	11,3	11,5	12,3	12,3	11,2
Data separated into sections 1-3										
Mode	19,0	19,0	18,5	18,5	19,0	20,5	19,5	19,0	19,0	19,5
Average	18,1	18,2	18,3	18,1	19,4	21,0	20,0	18,6	18,9	20,0
Min	11,0	10,0	12,0	11,5	15,5	10,0	11,0	12,0	12,0	14,0
Max	21,0	21,5	22,0	21,5	26,5	24,0	23,5	25,5	27,5	33,5
Std. dev.	1,6	1,8	1,3	1,3	1,4	2,6	2,4	1,4	1,5	2,5
Mode	17,0	17,5	17,5	23,5	24,0	23,5	24,0	21,5	21,5	23,0
Average	31,8	32,1	32,0	32,3	35,2	22,2	23,2	21,2	21,1	22,7
Min	16,5	17,0	16,5	16,5	16,5	16,5	13,5	16,5	16,0	17,0
Max	51,5	50,5	50,5	51,0	58,5	28,0	29,0	26,0	26,0	27,0
Std.dev.	10,1	9,5	10,9	11,4	13,7	2,9	3,2	2,2	2,3	2,5
Mode	32,5	34,5	32,0	32,0	32,0	50,0	49,5	49,5	49,5	50,0
Average	36,3	37,0	37,0	37,0	38,0	37,9	38,1	38,7	38,4	37,9
Min	19,5	19,5	18,0	18,5	13,5	21,5	19,5	19,5	19,5	13,0
Max	46,0	47,0	48,0	48,0	50,5	52,5	59,5	71,0	74,0	59,5
Std.dev.	5,3	5,4	6,2	6,2	7,2	10,1	10,3	10,6	10,9	9,8

Sections 1 and 2 are only around 1 week time, whereas section 3 data covers half of the experiment.

Figure A1. Composting experiments measured temperature data is presenting result from tunnel (T1 and T2), one-month windrow composting (A11 and A12) and last month data from four-month windrow composting (A2) experiments. Temperature data has been divided into sections between turn-arounds from which mode, average, minimum and maximum temperature and standard deviation has been reviewed. Color-coding has used for highlighting mesophilic, thermophilic and hygienization conditions.

References

1. Tilman, D.; Clark, M.; Williams, D.; Kimmel, K.; Polasky, S.; Packer, C. Future threats to biodiversity and pathways to their prevention. *Nature* **2017**, *546*, 73–81. [CrossRef]
2. Ehrenfeld, J.G. Ecosystem Consequences of Biological Invasions. *Annu. Rev. Ecol. Evol. Syst.* **2010**, *41*, 59–80. [CrossRef]
3. Powell, K.I.; Chase, J.M.; Knight, T.M. A synthesis of plant invasion effects on biodiversity across spatial scales. *Am. J. Bot.* **2011**, *98*, 539–548. [CrossRef]
4. Powell, K.I.; Chase, J.M.; Knight, T.M. Invasive Plants Have Scale-Dependent Effects on Diversity by Altering Species-Area Relationships. *Science* **2013**, *339*, 316–318. [CrossRef]
5. Ramula, S.; Pihlaja, K. Plant communities and the reproductive success of native plants after the invasion of an ornamental herb. *Biol. Invasions* **2012**, *14*, 2079–2090. [CrossRef]
6. Donald, P.F. Biodiversity Impacts of Some Agricultural Commodity Production Systems. *Conserv. Biol.* **2004**, *18*, 17–37. [CrossRef]
7. Hasler, K. Environmental Impact of Mineral Fertilizers: Possible Improvements through the Adoption of Eco-Innovations. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2017.

8. Tilman, D.; Socolow, R.; Foley, J.A.; Hill, J.; Larson, E.; Lynd, L.; Pacala, S.; Reilly, J.; Searchinger, T.; Somerville, C.; et al. Beneficial Biofuels—The Food, Energy, and Environment Trilemma. *Science* **2009**, *325*, 270–271. [CrossRef] [PubMed]
9. Immerzeel, D.J.; Verweij, P.A.; vad der Hilst, F.; Faaij, A.P.C. Biodiversity impacts of bioenergy crop production: A state-of-the-art review. *GCB Bioenergy* **2013**, *6*, 183–209. [CrossRef]
10. Hensgen, F.; Richter, F.; Wachendorf, M. Integrated generation of solid fuel and biogas from green cut material from landscape conservation and private households. *Bioresour. Technol.* **2011**, *102*, 10441–10450. [CrossRef]
11. Bucholc, K.; Szymczak-Żyła, M.; Lubecki, L.; Zamojska, A.; Hapter, P.; Tjernström, E.; Kowalewska, G. Nutrient content in macrophyta collected from southern Baltic Sea beaches in relation to eutrophication and biogas production. *Sci. Total Environ.* **2014**, *473–474*, 298–307. [CrossRef]
12. Oelmann, Y.; Broll, G.; Hölzel, N.; Kleinebecker, T.; Vogel, A.; Schwartze, P. Nutrient impoverishment and limitation of productivity after 20 years of conservation management in wet grasslands of north-western Germany. *Biol. Conserv.* **2009**, *142*, 2941–2948. [CrossRef]
13. Jakobsson, S.; Bernes, C.; Bullock, J.M.; Verheyen, K.; Lindborg, R. How does roadside vegetation management afect the diversity of vascular plants and invertebrates? A systematic review. *Environ. Evid.* **2018**, *7*, 17. [CrossRef]
14. Valtonen, A.; Jantunen, J.; Saarinen, K. Flora and lepidoptera fauna adversely affected by invasive *Lupinus polyphyllus* along road verges. *Biol. Conserv.* **2006**, *133*, 389–396. [CrossRef]
15. Kettenring, K.M.; Reinhardt Adams, C. Lessons learned from invasive plant control experiments: A systematic review and meta-analysis. *J. Appl. Ecol.* **2011**, *48*, 970–979. [CrossRef]
16. Ramula, S. Annual mowing has the potential to reduce the invasion of herbaceous *Lupinus polyphyllus*. *Biol. Invasions* **2020**, *22*, 3163–3173. [CrossRef]
17. Manninen, S.; Forss, S.; Venn, S. Management mitigates the impact of urbanization on meadow vegetation. *Urban. Ecosyst.* **2010**, *13*, 461–481. [CrossRef]
18. Reza, M.S.; Ahmed, A.; Caesarendra, W.; Abu Bakar, M.S.; Shams, S.; Saidur, R.; Aslfattahi, N.; Azad, A.K. *Acacia Holosericea*: An Invasive Species for Bio-char, Bio-oil, and Biogas Production. *Bioengineering* **2019**, *6*, 33. [CrossRef]
19. Joseph, B.; Hensgen, F.; Wachendorf, M. Life Cycle Assessment of bioenergy production from mountainous grasslands invaded by lupine (*Lupinus polyphyllus* Lindl.). *J. Environ. Manag.* **2020**, *275*, 111182. [CrossRef]
20. Schröder, P.; Beckers, B.B.; Daniels, S.; Gnädinger, F.; Maestri, E.; Marmiroli, N.; Mench, M.; Millan, R.; Obermeier, M.M.; Oustriere, N.; et al. Intensify production, transform biomass to energy and novel goods and protect soils in Europe—A vision how to mobilize marginal lands. *Sci. Total Environ.* **2018**, *616–617*, 1101–1123. [CrossRef]
21. Tafdrup, S. Viable energy production and waste recycling from anaerobic digestion of manure and other biomass materials. *Biomass Bioenergy* **1995**, *9*, 303–314. [CrossRef]
22. Grigatti, M.; Barbanti, L.; Hassan, M.U.; Ciavatta, C. Fertilizing potential and CO_2 emissions following the utilization of fresh and composted food-waste anaerobic digestates. *Sci. Total Environ.* **2020**, *698*, 134198. [CrossRef]
23. Cadena, E.; Colón, J.; Artola, A.; Sánchez, A.; Font, X. Environmental impact of two aerobic composting technologies using life cycle assessment. *Int. J. Life Cycle Assess.* **2009**, *14*, 401–410. [CrossRef]
24. Walling, E.; Vaneeckhaute, C. Novel simple approaches to modeling composting kinetics. *J. Environ. Chem. Eng.* **2021**, *9*, 105243. [CrossRef]
25. Sundberg, C.; Smårs, S.; Jönsson, H. Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. *Bioresour. Technol.* **2004**, *95*, 145–150. [CrossRef]
26. Rocamora, I.; Wagland, S.T.; Villa, R.; Simpson, E.W.; Fernández, O.; Bajón-Fernández, Y. Dry anaerobic digestion of organic waste: A review of operational parameters and their impact on process performance. *Bioresour. Technol.* **2020**, *299*, 122681. [CrossRef]
27. Ward, A.J.; Hobbs, P.J.; Holliman, P.J.; Jones, D.L. Optimisation of the anaerobic digestion of agricultural resources. *Bioresour. Technol.* **2008**, *99*, 7928–7940. [CrossRef] [PubMed]
28. Cioabla, A.E.; Ionel, I.; Dumitrel, G.A.; Popescu, F. Comparative study on factors affecting anaerobic digestion of agricultural vegetal residues. *Biotechnol. Biofuels* **2012**, *5*, 39. [CrossRef]
29. Bonthoux, S.; Brunc, M.; Di Pietro, F.; Greulich, S.; Bouché-Pillon, S. How can wastelands promote biodiversity in cities? A review. *Landsc. Urban. Plan.* **2014**, *132*, 79–88. [CrossRef]
30. Joly, M.; Bertrand, P.; Gbangou, R.Y.; White, M.-C.; Dube, J.; Lavoie, C. Paving the Way for Invasive Species: Road Type and the Spread of Common Ragweed (*Ambrosia artemisiifolia*). *Environ. Manag.* **2011**, *48*, 514–522. [CrossRef]
31. Westerman, P.R.; Hildebrandt, F.; Gerowitt, B. Weed seed survival following ensiling and mesophilic anaerobic digestion in batch reactors. *Weed Res.* **2012**, *52*, 286–295. [CrossRef]
32. Finch-Savage, W.E.; Footitt, S. Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. *J. Exp. Bot.* **2017**, *68*, 843–856. [CrossRef] [PubMed]
33. Chahtane, H.; Kim, W.; Lopez-Molina, L. Primary seed dormancy: A temporally multilayered riddle waiting to be unlocked. *J. Exp. Bot.* **2017**, *68*, 857–869. [CrossRef]
34. Dahlquist, R.M.; Prather, T.S.; Stapleton, J.J. Time and Temperature Requirements for Weed Seed Thermal Death. *Weed Sci.* **2007**, *55*, 619–625. [CrossRef]
35. Sahlström, L. A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresour. Technol.* **2003**, *87*, 161–166. [CrossRef]

36. Meier, E.J.; Waliczek, T.M.; Abbott, M. Large-Scale Composting as a Means of Managing Invasive Plants in the Rio Grande River Valley Basin. *Invasive Plant Sci. Manag.* **2014**, *7*, 473–482. [CrossRef]
37. Blumenthal, D.M.; LeCain, D.R.; Augustine, D.J. Composted manure application promotes long-term invasion of semi-arid rangeland by *Bromus tectorum*. *Ecosphere* **2017**, *8*, e01960. [CrossRef]
38. Pérez, E.A.; Téllez, T.R.; Maqueda, S.R.; Linares, P.J.C.; Pardo, F.M.V.; Medina, P.L.R.; Moreno, J.L.; Gallego, F.L.; Cortés, J.G.; Guzmán, J.M.S. Seed germination and risks of using the invasive plant Eichhornia crassipes (Mart.) Solms-Laub. (water hyacinth) for composting, ovine feeding and biogas production. *Acta Bot. Gallica Bot. Lett.* **2015**, *162*, 203–214. [CrossRef]
39. Ministry of Agriculture and Forestry in Finland. *Finland's National Strategy on Invasive Alien Species*; Ministry of Agriculture and Forestry: Helsinki, Finland, 2012.
40. Ministry of Agriculture and Forestry in Finland. *Management Plan to Prevent Invasive Species of National Concern*; Ministry of Agriculture and Forestry: Helsinki, Finland, 2020.
41. Fremstad, E. NOBANIS—Invasive Alien Species Fact Sheet *Lupinus polyphyllus*. Online Database of the European Network on Invasive Alien Species—NOBANIS 2010. Available online: https://www.nobanis.org/globalassets/speciesinfo/l/lupinus-polyphyllus/lupinus-polyphyllus.pdf (accessed on 3 May 2021).
42. Li, S.-L.; Vasemägi, A.; Ramula, S. Genetic variation and population structure of the garden escaper *Lupinus polyphyllus* in Finland. *Plant. Syst. Evol.* **2016**, *302*, 399–407. [CrossRef]
43. Jauni, M.; Ramula, S. Demographic mechanisms of disturbance and plant diversity promoting the establishment of invasive *Lupinus polyphyllus*. *J. Plant Ecol.* **2017**, *10*, 510–517.
44. Ramula, S. Linking vital rates to invasiveness of a perennial herb. *Oecologia* **2014**, *174*, 1255–1264. [CrossRef]
45. CABI. *Lupinus polyphyllus* (garden lupin). CABI Datasheet 2021. Available online: https://www.cabi.org/isc/datasheet/31710 (accessed on 15 March 2021).
46. Aniszewski, T.; Kupari, M.H.; Leinonen, A.J. Seed number, seed size and seed diversity in Washington lupin (*Lupinus polyphyllus* Lindl.). *Ann. Bot.* **2001**, *87*, 77–82. [CrossRef]
47. Klinger, Y.P.; Eckstein, R.L.; Horlemann, D.; Otte, A.; Ludewig, K. Germination of the invasive legume *Lupinus polyphyllus* depends on cutting date and seed morphology. *NeoBiota* **2020**, *60*, 79–95. [CrossRef]
48. Ludewik, K.; Hansen, W.; Klinger, Y.P.; Eckstein, R.L.; Otte, A. Seed bank offers potential for active restoration of mountain meadows. *Restor. Ecol.* **2020**, *29*, e13311. [CrossRef]
49. Sapra, R.L.; Narain, P.; Bhat, S.R.; Lal, S.K.; Jain, S.K. Prediction of seed longevity in the genebank: How reliable are the estimates? *Curr. Sci.* **2003**, *85*, 1612–1616.
50. Ramula, S.; Kalske, A. Introduced plants of *Lupinus polyphyllus* are larger but flower less frequently than conspecifics from the native range: Results of the first year. *Ecol. Evol.* **2020**, *10*, 13742–13751. [CrossRef]
51. Scott, D. Perennial or Russell lupin: A potential high country pasture legume. *Proc. N. Z. Grassl. Assoc.* **1989**, *50*, 203–206.
52. Villar, I.; Alves, D.; Garrido, J.; Mato, S. Evolution of microbial dynamics during the maturation phase of the composting of different types of waste. *Waste Manag.* **2016**, *54*, 83–92. [CrossRef]
53. Albihn, A.; Vinnerås, B. Biosecurity and arable use of manure and biowaste—Treatment alternatives. *Livest. Sci.* **2007**, *112*, 232–239. [CrossRef]
54. Mengqi, Z.; Shi, A.; Ajmal, M.; Ye, L.; Awais, M. Comprehensive review on agricultural waste utilization and high-temperature fermentation and composting. *Biomass Convers. Biorefinery* **2021**. [CrossRef]
55. Westerman, P.R.; Heiermann, M.; Pottberg, U.; Rodemann, B.; Gerowitt, B. Weed seed survival during mesophilic anaerobic digestion in biogas plants. *Weed Res.* **2012**, *52*, 307–316. [CrossRef]
56. Šarapatka, B.; Holub, M.; Lhotská, M. The Effect of Farmyard Manure Anaerobic Treatment on Weed Seed Viability. *Biol. Agric. Hortic. An Int. J. Sustain. Prod. Syst.* **1993**, *10*, 1–8. [CrossRef]
57. Johansen, A.; Nielsen, H.B.; Hansen, C.M.; Andreasen, C.; Carlsgart, J.; Hauggard-Nielsen, H.; Roepstorff, A. Survival of weed seeds and animal parasites as affected by anaerobic digestion at meso- and thermophilic conditions. *Waste Manag.* **2013**, *33*, 807–812. [CrossRef]
58. Zhou, L.; Hülsemann, B.; Merkle, W.; Guo, J.; Dong, J.; Piepho, H.-P.; Gerhards, R.; Müller, J.; Oechsner, H. Influence of Anaerobic Digestion Processes on the Germination of Weed Seeds. *Gesunde Pflanzen* **2020**, *72*, 181–194. [CrossRef]
59. Sarker, S.; Lamb, J.J.; Hjelme, D.R.; Lien, K.M. A Review of the Role of Critical Parameters in the Design and Operation of Biogas Production Plants. *Appl. Sci.* **2019**, *9*, 1915. [CrossRef]
60. Arfin-Khan, M.A.S.; Vetter, V.M.S.; Reshi, Z.A.; Dar, P.A.; Jentsch, A. Factors influencing seedling emergence of three global invaders in greenhouses representing major eco-regions of the world. *Plant Biol.* **2018**, *20*, 610–618. [CrossRef] [PubMed]
61. Otte, A.; Volz, H. *Lupinus polyphyllus* Bundesamt für Naturschutz. Neobiota.de. Gebietsfremde und invasive Arten in Deutschland. Available online: https://neobiota.bfn.de/handbuch/gefaesspflanzen/lupinus-polyphyllus.html (accessed on 4 May 2021).
62. Elliot, C.; Fischer, D.G.; LeRoy, C.J. Germination of Three Native *Lupinus* Species in Response to Temperature. *Northwest. Sci.* **2011**, *85*, 403–410. [CrossRef]