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Assessing Germinating Seeds of Legume and Cereal Crops to Enhance Oxygen Depletion: A Novel Approach in Hermetic Storage

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Assessing Germinating Seeds of Legume and Cereal Crops to Enhance Oxygen Depletion: A Novel Approach in Hermetic Storage

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Abstract: Hermetic storage systems are used around the world to reduce stored product losses. Scavenging residual oxygen in hermetic containers can further enhance their effectiveness in minimizing stored commodity losses. Our objective was to assess the effectiveness of germinating seeds of soybeans, rice, cowpeas, and corn in scavenging oxygen. There were six germination stages: seeds soaked for 24 h and allowed to grow for 1, 2, 3, 4, 5, or 6 days (T1 to T6). Oxygen consumption was monitored for 30 h. Root length, the weight of mobilized seed reserve, and visual fungal growth were also assessed. The results showed that cowpeas in their fourth (T4), fifth (T5), and sixth (T6) germination stages were the most effective in scavenging oxygen to below 5% after only 12 h. Corn in its fifth (T5) germination stage took twice the time (24 h) of cowpeas (T4–T6) to reach 5%. Hypoxia affected the growth of radicle length and seed reserve mobilization in all crops except soybeans. Very minimal fungal growth was observed on germinating cowpea seeds under hermetic conditions. The fourth stage (T4) of germinating cowpeas has more potential as an oxygen scavenger because it requires less time to grow, and the seeds are easy to handle. Further research is needed to understand the role of seed weight and sizes, crop varieties, and genetic mechanisms that govern rapid oxygen consumption by germinating seeds among crops. Harnessing seeds as oxygen scavengers to reduce storage losses holds the promise of advancing the sustainable utilization of resources on smallholder farms.

Keywords: residual oxygen; hermetic storage; legume and cereal seeds; oxygen scavenger

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1. Introduction

The postharvest loss of grains during storage is a major threat to food and nutritional security, especially in developing countries. Two primary sources of postharvest grain losses are biotic and abiotic factors. The biotic factors include insects, pests, rodents, and fungi, whereas abiotic ones include temperature, moisture content, relative humidity (R.H.), and the thermal properties of grains [1]. Insects alone can contribute 30–40% to postharvest loss in developing countries [2]. Thus, insects reduce the quality and quantity of grains, lowering their market value and resulting in economic losses [3].

Smallholder farmers use several approaches to protect grains, including pesticides [4]. However, misuse and excessive use of synthetic insecticides can lead to health and environmental risks and insect resistance [5,6]. Alternatives to chemical control methods include modified and controlled atmospheres, such as hermetic storage. Millions of smallholder farmers use hermetic storage (H.S.) methods (e.g., airtight bags, metal and plastic silos, etc.) to protect their stored grain against insect pest attacks. Hermetic storage methods work by minimizing air movement from the outside to the inside of the airtight container. Insects and other organisms consume the remaining oxygen inside the container through respiration. Low oxygen prevents insects from feeding, growing, and reproducing [7–9].

The process of oxygen depletion in hermetic storage is gradual and can extend over several days or weeks, contingent on factors such as the initial insect infestation level and the temperature [10,11]. This slow process of oxygen depletion carries the risk of causing additional grain damage or compromising food quality. Food quality becomes critical, particularly when preserving products such as biofortified maize. Biofortified maize, developed to address macro- and micronutrient deficiencies (e.g., provitamin A) among women and children in sub-Saharan Africa, Latin America, and Asia [12–14], is susceptible to losing its nutritional value in the presence of prolonged oxygen exposure [15]. Postharvest storage has been identified as a critical intervention to retain provitamin A carotenoids given their highly oxidative nature [16]. However, research has indicated that hermetic bags are ineffective in preventing the loss of provitamin A carotenoids during storage, primarily because of intergranular residual oxygen [17].

Various methods are used to improve the effectiveness of hermetic storage, including the utilization of oxygen scavengers, soaked seeds, or gases like nitrogen and carbon dioxide, to eliminate the residual oxygen [18–21]. Oxygen scavengers, in particular, can deplete residual oxygen to levels much lower than those achieved with hermetic storage alone, thereby contributing to the preservation of product quality [22]. Research findings on biofortified maize have indicated that PICS bags with oxygen scavengers retain more provitamin A carotenoids compared with grain stored in PICS bags alone or regular polypropylene woven bags [17,23]. Commercially available synthetic oxygen scavengers are used for various applications, including maintaining product quality, minimizing degradation, and extending shelf life [19,24]. However, these synthetic oxygen scavengers are expensive, unaffordable, and unavailable to smallholder farmers in developing countries.

This study explored germinating seeds as an alternative to commercially available oxygen scavengers. This research aimed to assess (i) the effectiveness of germinating seeds of two legumes (cowpeas and soybeans) and cereal (rice and corn) crop species at depleting oxygen in airtight storage containers and (ii) the impact of hypoxia on seed growth and reserve mobilization. The hypothesis for this study is twofold: (i) Germinating seeds of legumes (cowpeas and soybeans) and cereals (rice and corn) will exhibit varying capabilities in depleting oxygen within hermetic containers. We anticipate significant differences in their oxygen-scavenging capacities. (ii) Hypoxia, resulting from the oxygen-depleting action of germinating seeds, is expected to exert an influence on both seed growth and the mobilization of stored reserves. We anticipate that a reduced oxygen environment will trigger specific physiological responses in germinating seeds, potentially altering growth patterns and the utilization of stored reserves.

We posit that at least one crop species will effectively reduce oxygen levels within hermetic storage, potentially offering a viable alternative to commercially available oxygen scavengers. These findings could prove invaluable to smallholder farmers and other stakeholders in the grain value chain interested in mitigating pest damage and preserving the quality of commodities, thereby ensuring food and nutritional security on smallholder farms. Using germinating seeds as oxygen scavengers offers practical advantages to smallholder farmers. The availability of seeds on their farms makes it a feasible and accessible solution. The simplicity of the germination process, requiring minimal training, further enhances its appeal by reducing the learning curve for farmers. This approach not only aligns with the existing resources of smallholder farmers but also provides a straightforward and sustainable way to improve storage conditions.

2. Materials and Methods

The experiment was conducted in the Postharvest Innovation for Crop Storage laboratory (PICS) at Purdue University (West Lafayette, IN, USA) in 2022 and 2023.

2.1. Seed Supply and Preparation

Untreated seeds of soybeans (*Glycine max*), rice (*Oryza sativa*), black-eyed cowpeas (*Vigna unguiculata*), and corn (*Zea mays*) were used in this experiment. These crops, partic-

ularly cowpeas and corn, are commonly stored in hermetic bags by smallholder farmers in developing countries. Soybeans (variety Hutcheson, non-GMO) and rice (variety Rex, a non-GMO hybrid long grain) were purchased from Hancock Farm & Seed Company (Dade City, FL, USA), each with a germination rate of 80%. Similarly, cowpeas with (a germination rate of 77%) were obtained from L.A Hearne company (King City, CA, USA). Reid's yellow dent corn (germination rate of 92%), which is an open-pollinated variety, was purchased from Urban Farmer Seeds LLC (Westfield, IN, USA).

Seed preparation involved sorting to remove inert materials and any broken or damaged seeds. The seeds were individually weighed to standardize them and sorted into groups based on seed mass: small, medium, and large. The median-weight group, which had the highest number of seeds, was selected for the experiment, as approximately 1500 seeds were needed for each crop. The selected group had an initial seed weight of 180 ± 10, 29 ± 10, 290 ± 10, and 330 ± 10 mg/seed for soybeans, rice, cowpeas, and corn, respectively. To minimize the interference of microorganisms, rice, cowpea, and corn seeds were surface-sterilized in a 3.5% sodium hypochlorite solution for 15 min [25], while soybean seeds were sterilized in a 7.5% sodium hypochlorite solution for 1 min. Soybean seeds are sensitive to sodium hypochlorite, and prolonged exposure to this chemical can adversely impact their germination process. All seeds were subsequently rinsed with distilled water three times before the start of the germination process.

2.2. Seed Germination and Experimental Setup

After sterilization, 25 seeds were placed in 133 mL jars filled with 50 mL of distilled water for 24 h. There were ten jars for each treatment, resulting in 60 jars in total for six germinating stages (treatments). After imbibition, the seeds were allowed to germinate on the surface of two layers of Whatman no. 1 filter paper moistened with distilled water in 9 cm diameter sterile Petri dishes. The Petri dishes were kept in the dark at room temperature (22 ± 2 °C) for five days, and the filter paper was kept moist by adding distilled water as needed. The experiment comprised six treatments involving the soaking of seeds for 24 h followed by germination at different time points: (i) T1 = 1 day after imbibition, (ii) T2 = 2 days after imbibition, (iii) T3 = 3 days after imbibition, (iv) T4 = 4 days after imbibition, (v) T5 = 5 days after imbibition, and (vi) T6 = 6 days after imbibition.

A preliminary experiment was set up to identify the appropriate number of seeds and the jar volume required for the trial. It was found that five germinating seeds in a 55 mL hexagonal jar were suitable for the experiment (Figure 1). Each treatment had two storage conditions: hermetic and non-hermetic. In the hermetic condition, the jars were sealed with two layers of Parafilm M® Laboratory film outside the metallic lid, while in the non-hermetic condition, a perforated plastic lid was used. The non-hermetic condition was considered a control. There were ten replications for each combination of the six germination stages and two conditions, providing a total of 120 jars per crop species. Seeds in non-hermetic jars were sprinkled with distilled water through a cotton ball as needed. The experiment lasted for seven days.

2.3. Data Collection

2.3.1. Seed Moisture Content Determination

The initial moisture content for soybean, cowpea, and corn seeds was measured using an air-oven procedure in triplicate 15 g samples at 103 °C for 72 h [26]. For rice seeds, triplicate 15 g samples were dried in an oven at 130 °C for 16 h [27].

2.3.2. Oxygen, Temperature, and Relative Humidity (RH)

To monitor the oxygen level, fluorescent yellow OxyDots were attached to the inner walls of the jars. Oxygen levels were monitored for seven days using an OxySense® 525OI Oxygen Analyzer device (Industrial Physics, Devens, MA, USA). The oxygen concentrations were measured at intervals of 3 h for the first 12 h, then at 6 h intervals until 30 h, and then once daily for seven days. However, only data for the first 30 h were used to assess the

potential of germinating seeds as oxygen scavengers after some treatments reached anoxia (0%). A repeated measurement study design was used to record the changes in oxygen concentration in each treatment over time.

Figure 1. Hermetic jars containing five germinating seeds each.

The experiment was conducted in ambient room conditions, with temperature and RH monitored every hour using USB dataloggers (Lascar, Erie, PA, USA). Because of space constraints, the 55 mL jars could not accommodate dataloggers for recording the internal temperature and RH. Data on temperature and RH are presented in Figure S1.

2.3.3. Root Length and Weight of Mobilized Seed Reserve

Following seed soaking and germination to the appropriate treatment, 15 seedlings were taken from each Petri dish and divided into three groups. One group of five seedlings was placed in hermetic jars and another group in non-hermetic jars, and the remaining group was used to calculate the seedlings' dry weight and seed reserve utilization for the initial days. The root length was measured using a digital caliper before and after putting the germinating seeds in the hermetic and non-hermetic jars. The measured values for each set of five seeds were added together (given that seeds were wet and could not be labeled, and there were practical constraints on identifying each seed without marking) and rounded to the nearest whole number. To find the change in root length for a single seed, the initial measurement was subtracted from the final measurement and divided by five. After measuring the root length on the seventh day, the seedlings were carefully detached from the remaining part of the seed. Subsequently, the same seed was oven-dried to determine its dry weight. This measured dry weight of the seed remnant was then utilized to calculate the weight of the mobilized seed reserve (WMSR). The initial dry weight of the original seeds was determined using an oven-drying method, as described earlier, for all germinating seeds.

$$\text{Weight of Mobilized Seed Reserve } (\text{mg/mg}) = \frac{(IDW - DWSR) * 1000}{5 * IWS},$$

where [28,29]

IDW = Initial dry weight of original seed;
DWSR = Dry weight of seed remnant;
IWS = Initial weight (mg) of seeds.

The WMSR was calculated before the experimental setup (initial) and seven days later (final) for both hermetic and non-hermetic jars. The initial WMSR was calculated by subtracting the seed remnant's dry weight from the original seed's dry weight before placing them in the jars. After the seven-day experimental period, the WMSR was calculated for both hermetic (H-7d) and non-hermetic (NH-7d) jars. We standardized the WMSR of each crop (mg/mg) by dividing the calculated WMSR by the original seed weight of the

crop. This approach allowed us to assess the seed reserves utilized by the germinating seeds for seven days.

2.3.4. Microbial Growth

Germinating seeds and photographs of cowpeas infected with fungus were submitted to the Purdue Plant and Pest Diagnostic Lab (PPDL) (West Lafayette, IN, USA). Samples comprising four Petri dishes, each containing 25 seeds naturally infected with fungus, were collected before the experimental setup on the fifth day of germination. Additionally, photographs were collected on the seventh day of the experiment for each condition (hermetic and non-hermetic). Fungal spores were isolated from the collected samples, and slides were prepared by placing a single drop of sterile water on them. The slides were observed using two different microscopes: a Stemi 2000 C dissection microscope (C. Zeiss, Jena, Germany) and a Nikon Eclipse Ni (Nikon, Tokyo, Japan) compound microscope equipped with DCI illumination.

2.4. Statistical Analysis

2.4.1. Multiple Linear Regression Analysis

A multiple linear regression model was used to evaluate the effect of treatment (germination stage) and time on the oxygen depletion rate in hermetic jars of the four crops. A separate regression model was used for germinating seeds of each crop (i.e., soybeans, rice, cowpeas, and corn). The regression models for each crop included treatment (germinating seed stage), time, and the interaction term of treatment and time as explanatory variables in the model. Since the time variable data depicted a non-normal pattern, a log transformation of the time variable was performed before running the regression analysis.

The estimated multiple linear regression equation is expressed as follows:

$$\text{Oxygen concentration (\%)} = \beta_0 + \beta_1 \log(\text{time}) + \beta_2 \text{ treatment} + \beta_3 \log(\text{time}) * \text{treatment} + \varepsilon$$

where

β_0 = intercept;
β_1 = slope of log(time);
β_2 = slope of treatment;
β_3 = slope of interaction term of log(time) and treatment;
ε = error term.

A multiple regression model with interaction terms is

Oxygen concentration (%) = β_0 + β_1 log(time) + β_2 1(Trt = T1) + β_3 1(Trt = T2) + β_4 1(Trt = T3) + β_5 1(Trt = T4) + β_6 1(Trt = T5) + β_7 1(Trt = T6) + β_8 1(Trt = T1) log(time) + β_9 1(Trt = T2) log(time) + β_{10} 1(Trt = T3) log(time) + β_{11} 1(Trt = T4) log(time) + β_{12} 1(Trt = T5) log(time) + β_{13} 1(Trt = T6) log(time) + ε

1(Trt = T1) means it is 1 when treatment = T1; otherwise, it is 0.

$$\text{T1 } y = (\beta_0 + \beta_2) + (\beta_1 + \beta_8) \log(\text{time}) + \varepsilon$$

$$\text{T2 } y = (\beta_0 + \beta_3) + (\beta_1 + \beta_9) \log(\text{time}) + \varepsilon$$

$$\text{T3 } y = (\beta_0 + \beta_4) + (\beta_1 + \beta_{10}) \log(\text{time}) + \varepsilon$$

$$\text{T4 } y = (\beta_0 + \beta_5) + (\beta_1 + \beta_{11}) \log(\text{time}) + \varepsilon$$

$$\text{T5 } y = (\beta_0 + \beta_6) + (\beta_1 + \beta_{12}) \log(\text{time}) + \varepsilon$$

$$\text{T6 } y = (\beta_0 + \beta_7) + (\beta_1 + \beta_{13}) \log(\text{time}) + \varepsilon$$

After the regression analysis, a pairwise comparison analysis was performed using the R package "emmeans" to compare slope coefficients for all pairs of treatments [30]. The pairwise comparison of slope coefficients was conducted using Tukey adjustment at a

95% confidence level. In addition, a marginal slope for each treatment was estimated to calculate the amount of oxygen depleted per unit increase in exposure time.

2.4.2. Mean Comparison

Tukey's test was conducted to compare the means of oxygen concentration, changes in root length, and the weight of mobilized seed reserve. Means were separated using Tukey adjustments at a 95% confidence level. All statistical analyses were performed using the software package R version 4.2.2.

3. Results

3.1. Seed Moisture Content

The seed moisture contents (±standard error of mean) of soybeans, rice, cowpeas, and corn were 12.69 ± 0.11, 13.81 ± 0.04, 11.07 ± 0.12, and 13.27 ± 0.10% dry basis, respectively.

3.2. Oxygen Depletion

All crops exhibited different oxygen depletion behaviors. Oxygen depletion was affected by treatments ($F = 266.59$, $p < 0.001$) and time ($F = 416.47$, $p < 0.001$). Cowpeas and corn exhibited significantly higher oxygen depletion rates than rice or soybeans (Figure 2). Treatments T4, T5, and T6 for cowpeas consistently exhibited higher oxygen consumption compared with T1, T2, and T3 from 6 to 24 h (Table 1). On the other hand, there were significant differences in the average oxygen concentration (%) between treatments in corn with no clear pattern except the T5 treatment, which was consistently lower from 6 to 24 h (Table 1). The mean oxygen level fell to about 5% after 12 h in all treatments for cowpeas except T1, T2, and T3. However, only T5 reached around 5% in corn after 24 h. There were no changes in oxygen levels in the non-hermetic jars. No changes in oxygen levels were observed in rice, and none of the soybean treatments reached the 5% level after 30 h (Figure 2).

Table 1. Average oxygen concentration (%) depleted by germinating cowpea and corn seeds after 6, 12, and 24 h. Treatments (Trts) consisted of soaking seeds for 24 h followed by germination duration: T1 = 1 day, T2 = 2 days, T3 = 3 days, T4 = 4 days, T5 = 5 days, and T6 = 6 days.

Oxygen Concentration (%, Mean ± SEM)						
	Cowpeas			Corn		
Trt	**6 h**	**12 h**	**24 h**	**6 h**	**12 h**	**24 h**
T1	19.40 ± 0.38 aA *	17.87 ± 0.37 aA	14.57 ± 0.81 aB	21.29 ± 0.35 aA	19.86 ± 0.29 aB	14.88 ± 0.49 aC
T2	19.14 ± 0.24 aA	17.34 ± 0.39 aA	13.68 ± 0.38 aB	17.76 ± 0.64 bA	13.07 ± 0.74 bB	7.31 ± 0.62 bcC
T3	15.85 ± 0.52 bA	10.59 ± 1.01 bB	3.88 ± 1.00 bC	16.13 ± 0.37 bcA	12.51 ± 0.59 bB	9.11 ± 0.94 bC
T4	12.62 ± 0.35 cA	5.20 ± 0.68 cB	0.53 ± 0.33 cC	15.52 ± 0.53 cdA	11.36 ± 0.68 bcB	6.07 ± 0.75 bcC
T5	11.78 ± 0.35 cA	4.98 ± 0.65 cB	0.42 ± 0.21 cC	13.77 ± 0.37 dA	9.16 ± 0.77 cB	5.22 ± 1.14 cC
T6	12.24 ± 0.44 cA	5.15 ± 0.64 cB	0.33 ± 0.15 cC	15.37 ± 0.24 cdA	11.55 ± 0.37 bcB	8.23 ± 0.84 bcC

* Under the same crop, means within the same column (lowercase letter) and the same row (uppercase letter) followed by the same letter are not significantly different ($p < 0.05$).

The multiple linear regression model results also support the findings in Figure 2 and Table 1. The slope estimates from 0 to 30 h revealed a significant interaction between exposure time and germination stages for all crops except rice and the non-hermetic (control) jars. A regression equation was statistically significant ($F = 137.3$, $p < 0.001$) with an R^2 of 78.89% for cowpeas. Cowpeas had the highest slope coefficients in T4, T5, and T6 (Table 2). However, no significant differences in slope coefficients were observed between these three treatments. A marginal slope estimate was generated by plugging the data from Table 2 into the previously described equation for each treatment. This estimate reveals that the hermetic storage receiving germinating cowpea seeds at the fourth, fifth, and sixth stages are expected to experience a reduction in oxygen levels by −1.77, −1.72, and

−1.80 units for every additional hour of exposure, respectively. A follow-up experiment to assess differences in the T4, T5, and T6 treatments of the germinating cowpea seeds was conducted using 110 mL jars (double the volume of the previous experiment). No significant differences in oxygen depletion or slope coefficients were observed between the three treatments.

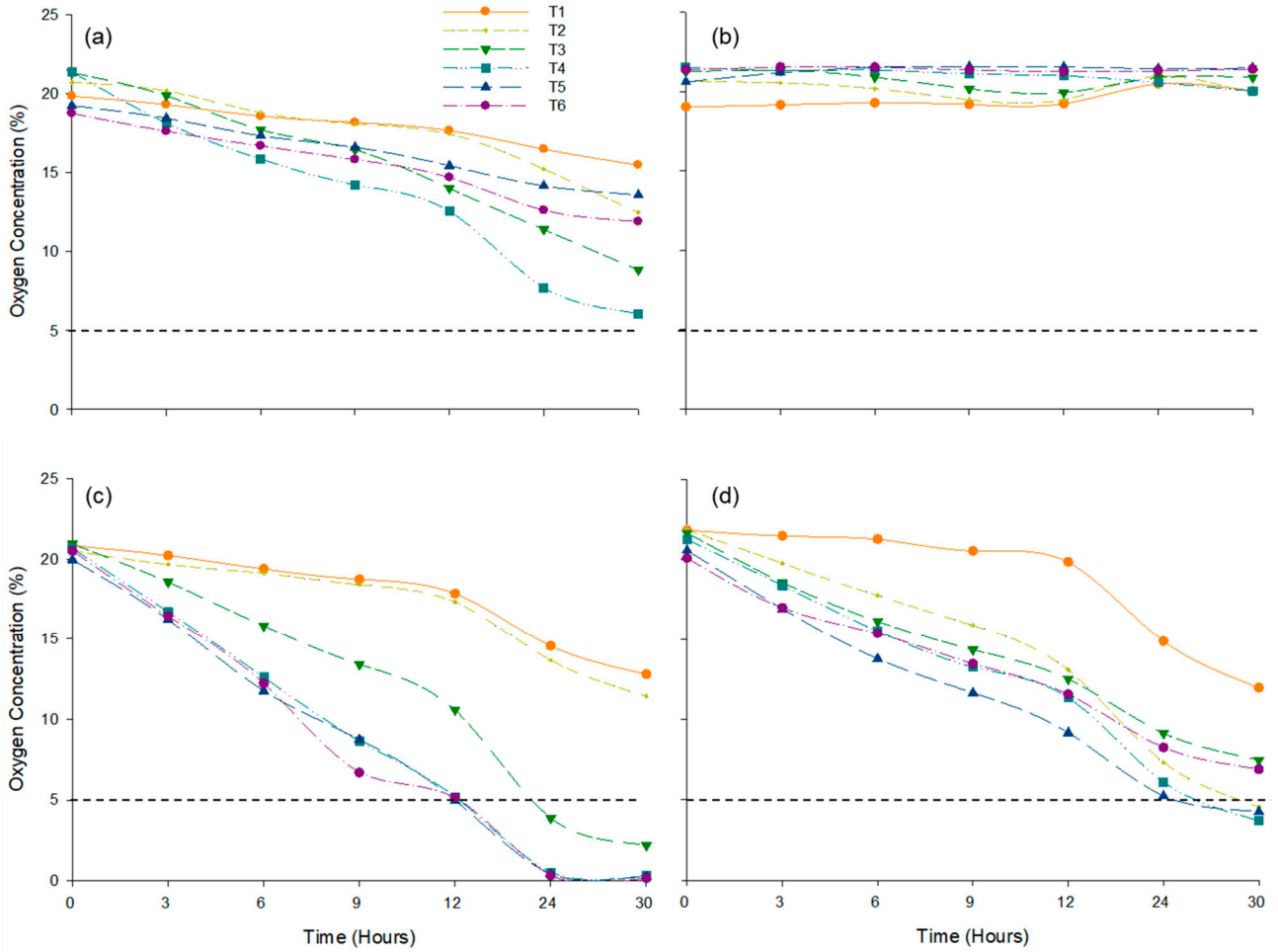

Figure 2. Oxygen depletion caused by germinated seeds of (**a**) soybeans, (**b**) rice, (**c**) cowpeas, and (**d**) corn under hermetic conditions for 30 h. Treatments consisted of soaking seeds for 24 h followed by germination duration: T1 = 1 day, T2 = 2 days, T3 = 3 days, T4 = 4 days, T5 = 5 days, and T6 = 6 days.

Table 2. Intercepts and slopes for regression equations of T4, T5, and T6 and interaction with log(time) for T4, T5, and T6 treatments of cowpea. Treatments consisted of soaking seeds for 24 h followed by germination duration: T4 = 4 days, T5 = 5 days, and T6 = 6 days.

Variables *	Estimate	Std. Error	t-Value	Pr(>|t|)
(Intercept) (β_0)	20.85	0.38	54.85	<0.001
log_hr (β_1)	0.04	0.11	0.35	0.72
T4 (β_5)	−9.81	0.54	−18.26	<0.001
T5 (β_6)	−10.20	0.54	−18.97	<0.001
T6 (β_7)	−10.23	0.54	−19.02	<0.001
log_hr: T4 (β_{11})	−1.77	0.15	−11.48	<0.001
log_hr: T5 (β_{12})	−1.72	0.15	−11.11	<0.001
log_hr: T6 (β_{13})	−1.80	0.15	−11.67	<0.001

* Variables consisted of germinated seeds: β_0 = intercept, β_1 = slope of log(time), β_5 = slope of T4, β_6 = slope of T5, β_7 = slope of T6, β_{11} = slope of T4 and log(time) interaction, β_{12} = slope of T5 and log(time) interaction, β_{13} = slope of T6 and log(time) interaction.

3.3. Effect of Hermetic Storage on Root Length

After seven days, seeds maintained under non-hermetic (control) conditions had relatively more developed roots than those kept under hermetic conditions (Figures 3–5). For rice, the change in root length was consistently higher in the non-hermetic (control) compared with hermetic storage. Hermetic storage had the most significant impact on rice, as no changes in root length were observed in each treatment (Figure 3b). For the other crops, no change in root length was observed between the control and hermetic storage except in treatment T1 for soybeans; T2, T3, and T5 for cowpeas; and T1, T2, T3, and T4 for corn (Figure 3a,c,d).

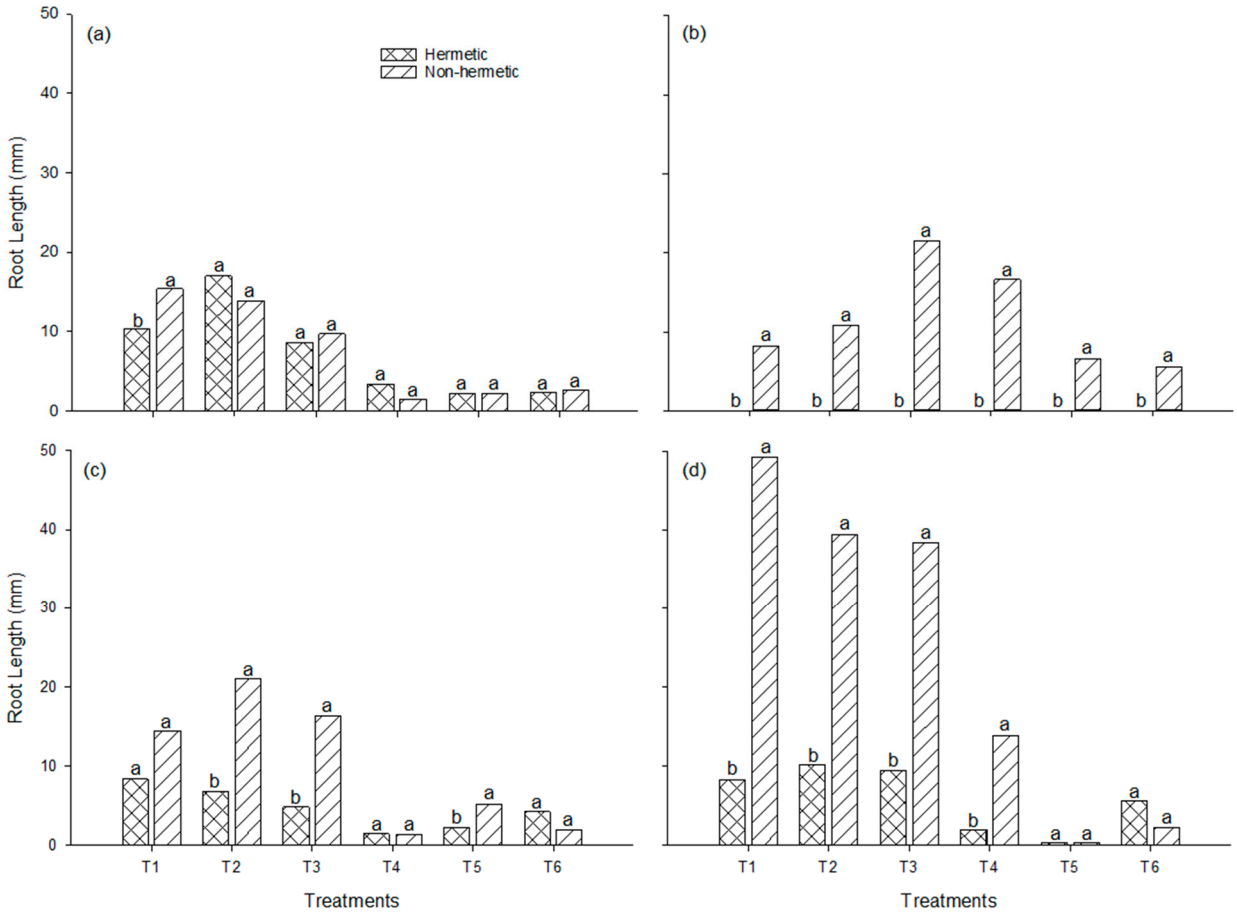

Figure 3. Change in root length (mm) of germinated seeds of (**a**) soybeans, (**b**) rice, (**c**) cowpeas, and (**d**) corn after seven days under hermetic and non-hermetic (control) conditions. Treatments consisted of soaking seeds for 24 h followed by germination duration: T1 = 1 day, T2 = 2 days, T3 = 3 days, T4 = 4 days, T5 = 5 days, and T6 = 6 days. Under the same crop and within the same treatment, letters indicate significant differences at 0.05.

3.4. Effect of Hermetic Storage on the Weight of Mobilized Seed Reserve

The storage method (hypoxia) significantly impacted the mobilization of seed reserves in rice ($F = 146.46$, $p < 0.001$), cowpeas ($F = 46.69$, $p < 0.001$), and corn ($F = 293.71$, $p < 0.001$) but not in soybeans ($F = 0.6$, $p < 0.439$) (Table 3). There was a trend of increasing weight in the mobilized seed reserve (WMSR) from T1 to T6 at the initial stage for all crops except rice. However, significant differences between treatments (T1 to T6) in hermetic storage were only seen in cowpeas and corn. When comparing hermetic to non-hermetic storage, there were no variations in the WMSR of the soybean treatments, but both storage methods varied from initial readings only in T6. In rice, the WMSR in hermetic storage was similar to those observed at the initial stage, but somewhat greater changes were seen in non-hermetic

storage within each treatment. In non-hermetic conditions, rice had a relatively identical or higher-weight mobilized seed reserve than soybeans, even though rice has a sixth of the soybean's weight. For corn and cowpeas, the WMSR was higher in non-hermetic conditions, followed by hermetic conditions, and then by the initial stage, except in the T2 treatment of cowpeas. Overall, cowpeas had the greatest seed reserve mobilization when all four crops were considered.

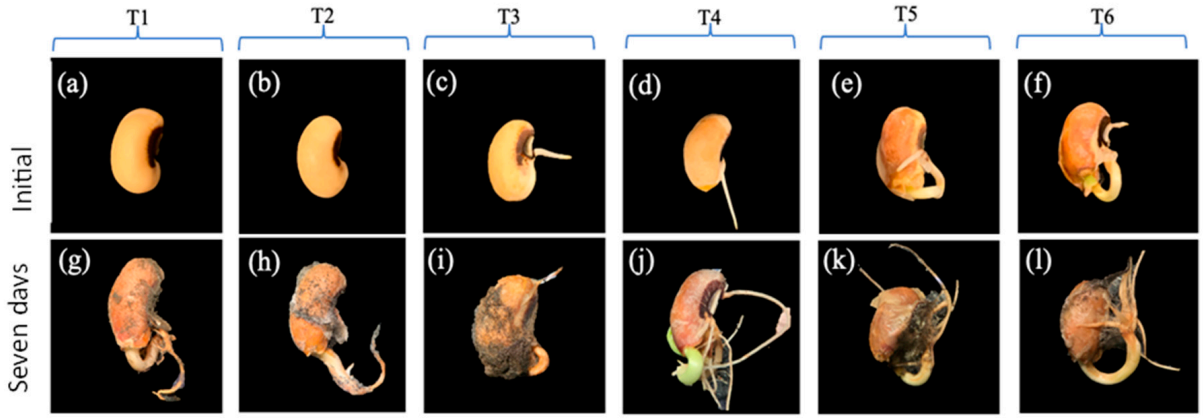

Figure 4. Pictures of cowpea seedlings at the initial (**a**–**f**) stage and seven days later (**g**–**l**) under non-hermetic conditions. Treatments consisted of soaking seeds for 24 h followed by germination duration: T1 = 1 day, T2 = 2 days, T3 = 3 days, T4 = 4 days, T5 = 5 days, and T6 = 6 days.

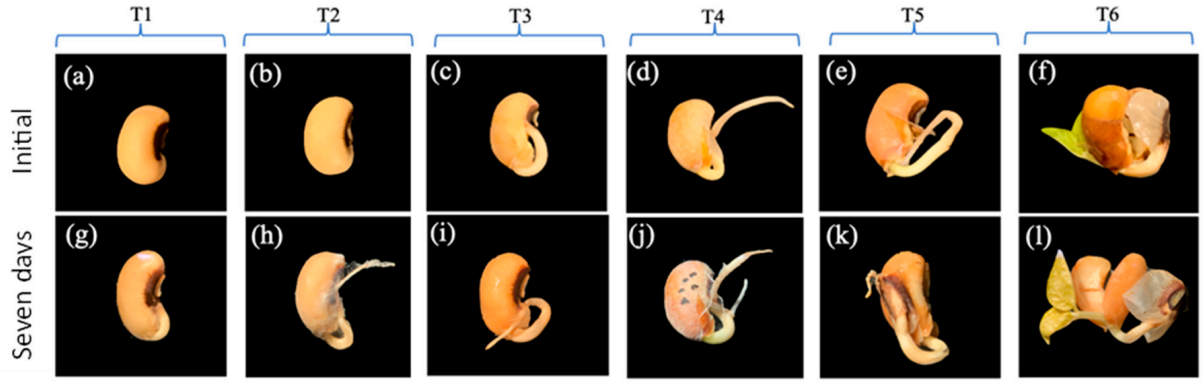

Figure 5. Pictures of cowpea seedlings at the initial (**a**–**f**) stage and seven days later (**g**–**l**) under hermetic conditions. Treatments consisted of soaking seeds for 24 h followed by germination duration: T1 = 1 day, T2 = 2 days, T3 = 3 days, T4 = 4 days, T5 = 5 days, and T6 = 6 days.

3.5. Microbial Growth

Cowpeas had the highest fungal activities in comparison with the other crops. Fungal growth was observed in all cowpea treatments kept in non-hermetic jars but was less in those in hermetic storage (Figure 6). No sporulation was visible on rice, corn, or soybeans in either hermetic or non-hermetic conditions. However, the germinating cowpea seeds stored in non-hermetic jars turned black, and the cotton balls inside the jars were completely covered with fungus. The germinating cowpea seeds stored in hermetic jars smelled of fermentation. *Rhizopus* spp. were identified from the seed samples. In addition, *Aspergillus* spp. and *Penicillium* spp. were identified from the photograph samples.

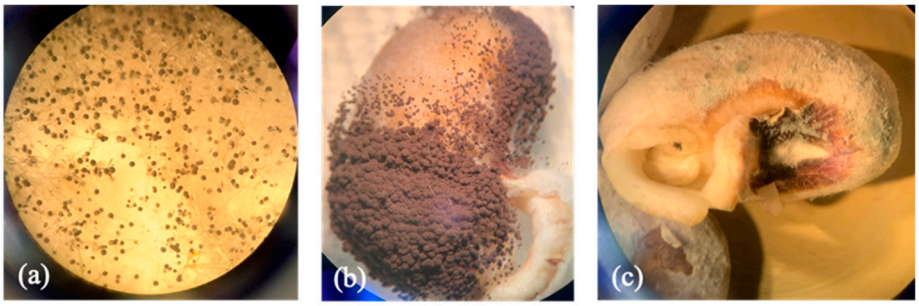

Figure 6. Different types of fungus were observed in cowpea seeds during the experiment: (**a**) *Rhizopus* spp. from T1 control (non-hermetic), (**b**) *Aspergillus* spp. from T2 control (non-hermetic), and (**c**) *Penicillium* spp. from T1 hermetic. Treatments consisted of soaking seeds for 24 h followed by germination duration: T1 = 1 day and T2 = 2 days.

Table 3. Weight of mobilized seed reserve (mg/mg) of germinated soybean, rice, cowpea, and corn seeds on the day of the experimental setup (initial) and 7 days after storage in hermetic (H-7d) and non-hermetic (NH-7d) jars. Treatments (Trts) consisted of soaking seeds for 24 h followed by germination duration: T1 = 1 day, T2 = 2 days, T3 = 3 days, T4 = 4 days, T5 = 5 days, and T6 = 6 days.

Mean Weight of Mobilized Seed Reserve (mg/mg ± SEM)						
	Soybeans			**Rice**		
Trt	**Initial**	**H-7d**	**NH-7d**	**Initial**	**H-7d**	**NH-7d**
T1	0.05 ± 0.008 cB *	0.10 ± 0.008 aA	0.11 ± 0.006 aA	0.01 ± 0.011 aB	0.02 ± 0.008 aB	0.06 ± 0.011 cA
T2	0.03 ± 0.006 cB	0.10 ± 0.006 aA	0.11 ± 0.011 aA	0.07 ± 0.007 aB	0.02 ± 0.007 aB	0.09 ± 0.010 bcA
T3	0.06 ± 0.006 bcB	0.10 ± 0.007 aA	0.12 ± 0.011 aA	−0.01 ± 0.017 aB	0.01 ± 0.007 aB	0.14 ± 0.011 abA
T4	0.06 ± 0.007 abcB	0.12 ± 0.006 aA	0.10 ± 0.007 aA	0.02 ± 0.009 aB	0.01 ± 0.009 aB	0.14 ± 0.016 abA
T5	0.08 ± 0.009 abA	0.11 ± 0.009 aA	0.11 ± 0.009 aA	0.03 ± 0.007 aB	0.03 ± 0.008 aB	0.15 ± 0.013 aA
T6	0.09 ± 0.007 aB	0.12 ± 0.008 aA	0.13 ± 0.008 aA	0.03 ± 0.013 aB	0.03 ± 0.011 aB	0.16 ± 0.010 aA
	Cowpeas			**Corn**		
Trt	**Initial**	**H-7d**	**NH-7d**	**Initial**	**H-7d**	**NH-7d**
T1	0.07 ± 0.004 cC	0.20 ± 0.004 bB	0.28 ± 0.016 abA	0.01 ± 0.003 dC	0.06 ± 0.003 eB	0.15 ± 0.007 cA
T2	0.08 ± 0.005 cB	0.22 ± 0.005 abA	0.25 ± 0.015 bA	0.02 ± 0.003 cdC	0.08 ± 0.004 deB	0.17 ± 0.010 bcA
T3	0.09 ± 0.006 cC	0.23 ± 0.010 abB	0.29 ± 0.017 abA	0.03 ± 0.006 cC	0.11 ± 0.005 cdB	0.20 ± 0.011 bA
T4	0.09 ± 0.003 cC	0.21 ± 0.006 abB	0.33 ± 0.034 abA	0.06 ± 0.004 bC	0.11 ± 0.007 cB	0.25 ± 0.013 aA
T5	0.12 ± 0.006 bC	0.23 ± 0.008 abB	0.30 ± 0.022 abA	0.08 ± 0.004 aC	0.18 ± 0.009 bB	0.27 ± 0.009 aA
T6	0.15 ± 0.006 aC	0.24 ± 0.010 aB	0.36 ± 0.041 aA	0.09 ± 0.005 aC	0.23 ± 0.012 aB	0.29 ± 0.008 aA

* Under the same crop, means within the same column (lowercase letter) and the same row (uppercase letter) followed by the same letter are not significantly different ($p > 0.05$).

4. Discussion

4.1. Oxygen Depletion by Various Crops

The findings of this study suggest that there is potential for using germinating seeds to deplete oxygen in hermetic containers. Except for rice, all crops tested showed gradual oxygen depletion at varying rates. Cowpeas and corn exhibited the fastest depletion rates, while soybeans had a comparatively slow rate. Of all the germinating seeds, cowpeas were the most efficient at depleting oxygen in a hermetic environment. This may be explained by the fact that the oxygen requirement for respiration during seed germination varies across plant species [31]. Respiration during germination varies based on seed reserves, such as starch, protein, lipids, and carbohydrate content. This sensitivity occurs because of the amount of oxygen required to break down these seed reserves for germination.

Dicot seeds, such as cowpeas and soybeans, have higher fat and/or protein content than monocot seeds and require more oxygen during germination [32]. Though soybean seeds contain more protein than lipids, they utilize lipids as an energy source for ger-

mination compared to protein [33]. Despite soybeans having higher protein and lipid content than cowpeas, surprisingly, our results showed that cowpea seeds depleted oxygen faster. This unexpected finding suggests that factors beyond seed reserve content, such as genetic differences, might explain these differences. Each plant species possesses unique adaptations and physiological traits that can influence its behavior under hypoxic conditions during germination. Investigating genetic factors that might explain why cowpea seeds exhibited faster oxygen depletion than soybeans is imperative. Unlocking specific genetic traits can streamline/accelerate the identification of crops or varieties with superior oxygen-scavenging capacities.

Besides seed composition, the four crops varied in seed size and weight. For instance, the average cowpea seed weighed 10 and 1.62 times more than rice and soybean seeds, respectively, but only 0.88 times of a corn seed. Since corn seeds did not deplete oxygen faster than cowpea seeds (despite weighing more), we assumed that seed weight had a minimal impact on oxygen depletion across plant families. Hence, we did not adjust the oxygen concentration based on the seed weight of crops during the analysis. When comparing cowpeas and soybeans, weight might contribute to oxygen depletion, as cowpea seeds weighed 1.62 times more than soybean seeds. Future studies should investigate the role of varying seed weights and sizes on oxygen depletion within the same crop family (e.g., legumes, cereals, etc.).

4.2. Oxygen Depletion via Germinating Seed Stages

Different stages of germinating seeds had varying levels of oxygen depletion. Oxygen levels below 5% effectively control stored product pests [3,7]. So, the germinating stages that reached a 5% level could be used to deplete oxygen. In cowpeas, T4, T5, and T6 depleted oxygen to 5% within 12 h, while T3 took 24 h to reach that level. On the other hand, in corn, only treatment T5 reached 5% in 24 h, and none of the soybean treatments reached that level even after 30 h. Stages of germinating seeds are known to have different requirements for oxygen consumption. Variations in oxygen depletion among germination stages may be due to the physiological changes inside the seeds during growth [34]. While this experiment was conducted under ambient conditions, it is essential to acknowledge that different temperatures can significantly influence the respiration process throughout the germination phases [35–37]. Future research efforts should explore the effect of temperature on germinating seed stages of promising crops such as cowpeas and resulting oxygen depletion.

4.3. Effects of Hypoxia on Radicle Growth and Weight of Mobilized Seed Reserve

The hypoxia created by the hermetic environment affected both the development of root length and WMSR in all crops except for soybeans. The hermetic environment significantly suppressed the growth of corn and rice seed roots. Other studies have demonstrated that germinating seeds under hypoxia have shorter root lengths [32,38]. Our findings suggest that hypoxia had a minimal effect on the root growth of cowpeas after the fourth germination stage (T4). This might explain why we observed few differences in oxygen depletion in cowpeas between the T4, T5, and T6 germination stages. The suppression of root growth in rice under hermetic conditions indicates low oxygen uptake by all the stages of the germinating seeds.

During germination, the development of the root–shoot axis is related to the reserve materials present in the cotyledon or endosperm of the seeds. These reserves are mainly translocated into the root–shoot axis during germination, where they are utilized both as a substrate for respiration and growth [34]. For instance, in cowpeas, approximately 26% of the initial protein is utilized within 24 to 48 h after soaking for radicle elongation, while 33.5% is mobilized between 48 and 72 h during hypocotyl expansion [39]. The initial radicle elongation is dependent on rapid protein reserve degradation between 48 and 120 h and is utilized for axis growth. After 144 h, protease activity ceases because of substrate depletion. Because of this, germinating seeds will not have enough energy to continue the

formation of the root–shoot axis, which might be the reason for minimal changes in radicle lengths after T4 and the lower mobilization of seed reserve in hermetic compared with non-hermetic conditions.

4.4. Hypoxia and Fungal Development

Fungal growth on high-moisture seeds (e.g., germinating seeds) stored in non-hermetic containers is expected and has been documented. Similarly, corn with high moisture content (e.g., 21% or above) stored in non-hermetic containers can experience fungal mycelia growth and mold sporulation [40,41]. These studies also demonstrated that wet maize stored in hermetic containers had little mold growth but smelled of fermentation. In this study, however, we only found evidence of fermentation in cowpeas. To effectively utilize germinating seeds as oxygen scavengers, it is imperative to assess their influence on the overall relative humidity and how this, in turn, affects the moisture content of the stored grain in hermetic containers. It becomes paramount to explore strategies to mitigate the release of humidity by germinating seeds to minimize its effect on the stored grain quality.

It is noteworthy that surface sterilization, while effective to a certain extent, does not entirely eradicate endophytic microorganisms [42]. This might be the source of microbial activities observed in germinating cowpea seeds. To mitigate these potential effects, the precise identification of microorganisms on seeds and appropriate sterilization methods are crucial during germination. While morphological traits offer initial insights into species identification, a more in-depth analysis using molecular techniques, such as DNA sequencing, becomes imperative for precise confirmation [43]. The presence of fungi during the initial stages of germination could have a lasting impact on oxygen consumption [44]. Though minimal on germinating cowpea seeds under hermetic storage, fungal growth increased with the germination stage (T6 > T5 > T4). Nevertheless, our data indicate minimal or no impact from fungal presence on oxygen consumption, as evidenced by the absence of statistical differences between T4, T5, and T6 at the end of the storage periods (Table 1).

It is crucial to proceed with caution when employing germinating seeds for oxygen depletion in hermetic storage alongside dry grain. The potential impact of mixing germinating seeds with stored commodities lies in the high moisture content of the former, which could adversely affect the quality of the stored grain. To offset any potential effects on stored product quality, isolating the germinating seeds within a small container (e.g., vials or small plastic containers) is imperative. This isolation is critical to preventing direct contact between the high-moisture germinating seeds and the stored grains. Such a precautionary measure is vital for minimizing the risk of grain contamination due to microorganisms and ensuring food safety during hermetic storage.

5. Conclusions

The findings of this study indicate that oxygen depletion caused by germinating seeds varies by crop and germinating stages. Germinating cowpea seeds can be used as oxygen scavengers in hermetic storage containers. Germinating cowpea seeds from the fourth stage (T4, T5, and T6) are effective at depleting oxygen to 5% in hermetic containers within 12 h. We recommend the cowpea's fourth germinating stage (T4) because it requires fewer days to achieve, has minimal fungal growth, and the seeds are easier to handle. To refine the use of germinating seeds as oxygen scavengers, further research is needed to (i) assess oxygen depletion caused by germinating seeds of various sizes and weights within the same crop family (e.g., cowpeas, chickpeas, soybeans, peas, and lentils), (ii) test the potential of oxygen depletion caused by different varieties within each crop (e.g., early compared with late-maturing varieties), (iii) investigate genetic factors that might explain why some crops exhibit faster oxygen depletion than others, (iv) determine the effect of temperature on germinating seeds and resulting oxygen depletion, (v) research ways to mitigate the release of humidity caused by germinating seeds into the stored grains, (vi) conduct microorganism

analysis using molecular techniques including DNA sequencing, and (vii) explore the effect of hypoxia induced by germinating seeds on insect mortality and grain quality.

Leveraging seeds as oxygen scavengers in hermetic storage can improve its efficacy and reduce storage losses on smallholder farms. Integrating germinating seeds as oxygen scavengers is a comprehensive strategy beyond storage solutions. This holistic approach addresses not only food loss but also preserves the nutritional value of crops during storage. The aim is to promote the sustainable utilization of resources and enhance the overall resilience of smallholder farmers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su152316403/s1, Figure S1. Prevailing ambient room temperature and relative humidity during the experiment on oxygen depletion by germinated seeds of cowpea, rice, soybean, and corn (23 August to 22 September 2022).

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References

1. Abedin, M.Z.; Rahman, M.Z.; Mia, M.I.A.; Rahman, K.M.M. In-store losses of rice and ways of reducing such losses at farmers' level: An assessment in selected regions of Bangladesh. *J. Bangladesh Agril. Univ.* **2012**, *10*, 133–144. [CrossRef]
2. Kumar, D.; Kalita, P. Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. *Foods* **2017**, *6*, 8. [CrossRef] [PubMed]
3. Baoua, I.B.; Amadou, L.; Ousmane, B.; Baributsa, D.; Murdock, L.L. PICS bags for post-harvest storage of maize grain in West Africa. *J. Stored Prod. Res.* **2014**, *58*, 20–28. [CrossRef]
4. Baoua, I.B.; Amadou, L.; Margam, V.; Murdock, L.L. Comparative evaluation of six storage methods for postharvest preservation of cowpea grain. *J. Stored Prod. Res.* **2012**, *49*, 171–175. [CrossRef]
5. Anaduaka, E.G.; Uchendu, N.O.; Asomadu, R.O.; Ezugwu, A.L.; Okeke, E.S.; Chidike Ezeorba, T.P. Widespread use of toxic agrochemicals and pesticides for agricultural products storage in Africa and developing countries: Possible panacea for ecotoxicology and health implications. *Heliyon* **2023**, *9*, e15173. [CrossRef] [PubMed]
6. Opit, G.P.; Phillips, T.W.; Aikins, M.J.; Hasan, M.M. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. *J. Econ. Entomol.* **2012**, *105*, 1107–1114. [CrossRef]
7. Murdock, L.L.; Margam, V.; Baoua, I.; Balfe, S.; Shade, R.E. Death by desiccation: Effects of hermetic storage on cowpea bruchids. *J. Stored Prod. Res.* **2012**, *49*, 166–170. [CrossRef]
8. Hoback, W.W.; Stanley, D.W. Insects in hypoxia. *J. Insect Physiol.* **2001**, *47*, 533–542. [CrossRef]
9. Navarro, S. Commercial applications of oxygen depleted atmospheres for the preservation of food commodities. In *Case Studies in Novel Food Processing Technologies*; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing Limited: Cambridge, UK, 2010; pp. 321–350, ISBN 9781845695514.
10. Donga, T.K.; Baributsa, D. Effect of temperature and insect infestation levels on oxygen depletion in hermetic storage. *Insects* **2023**, *14*, 621. [CrossRef]

11. Njoroge, A.W.; Affognon, H.D.; Mutungi, C.M.; Manono, J.; Lamuka, P.O.; Murdock, L.L. Triple bag hermetic storage delivers a lethal punch to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in stored maize. *J. Stored Prod. Res.* **2014**, *58*, 12–19. [CrossRef]
12. Goredema-Matongera, N.; Ndhlela, T.; Magorokosho, C.; Kamutando, C.N.; van Biljon, A.; Labuschagne, M. Multinutrient Biofortification of Maize (*Zea mays* L.) in Africa: Current Status, Opportunities and Limitations. *Nutrients* **2021**, *13*, 1039. [CrossRef] [PubMed]
13. Manjeru, P.; Van Biljon, A.; Labuschagne, M. The development and release of maize fortified with provitamin A carotenoids in developing countries. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 1284–1293. [CrossRef] [PubMed]
14. Nkhata, S.G.; Chilungo, S.; Memba, A.; Mponela, P. Biofortification of maize and sweetpotatoes with provitamin A carotenoids and implication on eradicating vitamin A deficiency in developing countries. *J. Agric. Food Res.* **2020**, *2*, 100068. [CrossRef]
15. Bechoff, A.; Chijioke, U.; Tomlins, K.I.; Govinden, P.; Ilona, P.; Westby, A.; Boy, E. Carotenoid stability during storage of yellow gari made from biofortified cassava or with palm oil. *J. Food Compos. Anal.* **2015**, *44*, 36–44. [CrossRef]
16. Mugode, L.; Ha, B.; Kaunda, A.; Sikombe, T.; Phiri, S.; Mutale, R.; Davis, C.; Tanumihardjo, S.; De Moura, F.F. Carotenoid Retention of Biofortified Provitamin A Maize (*Zea mays* L.) after Zambian Traditional Methods of Milling, Cooking and Storage. *J. Agric. Food Chem.* **2014**, *62*, 6317–6325. [CrossRef]
17. Taleon, V.; Mugode, L.; Cabrera-Soto, L.; Palacios-Rojas, N. Carotenoid retention in biofortified maize using different post-harvest storage and packaging methods. *Food Chem.* **2017**, *232*, 60–66. [CrossRef]
18. Kandel, P.; Scharf, M.E.; Mason, L.J.; Baributsa, D. Effect of hypoxia on the lethal mortality time of adult *Sitophilus oryzae* L. *Insects* **2021**, *12*, 952. [CrossRef]
19. Souza, R.; Peruch, G.; Santos Pires, A.C. dos Oxygen Scavengers: An Approach on Food Preservation. In *Structure and Function of Food Engineering*; InTech: Rijeka, Croatia, 2012; pp. 21–42. [CrossRef]
20. Navarro, S. The use of modified and controlled atmospheres for the disinfestation of stored products. *J. Pest Sci.* **2012**, *85*, 301–322. [CrossRef]
21. Taher, H.; Bartosik, R. Hermetic storage of dry soybean (Glycine max): Creating an effective modified atmosphere using soaked grain as O_2 depletor. In Proceedings of the 12th International Working Conference on Stored Product Protection (IWCSPP), Berlin, Germany, 7–11 October 2018; Volume 2, pp. 666–671.
22. Dey, A.; Neogi, S. Oxygen scavengers for food packaging applications: A review. *Trends Food Sci. Technol.* **2019**, *90*, 26–34. [CrossRef]
23. Nkhata, S.G.; Ortiz, D.; Baributsa, D.; Hamaker, B.; Rocheford, T.; Ferruzzi, M.G. Assessment of oxygen sequestration on effectiveness of Purdue Improved Crop Storage (PICS) bags in reducing carotenoid degradation during post-harvest storage of two biofortified orange maize genotypes. *J. Cereal Sci.* **2019**, *87*, 68–77. [CrossRef]
24. Cichello, S.A. Oxygen absorbers in food preservation: A review. *J. Food Sci. Technol.* **2015**, *52*, 1889–1895. [CrossRef]
25. Oyebanji, O.B.; Nweke, O.; Odebunmi, O.; Galadima, N.B.; Nnodi, U.N.; Afolabi, A.S.; Ogbadu, G.H. Simple, effective and economical explant-surface sterilization protocol for cowpea, rice and sorghum seeds. *African J. Biotech.* **2009**, *8*, 5395–5399.
26. *ASAE Standards D245.6*; Moisture Relationships of Plant-Based Agricultural Products. American Society of Agricultural and Biological Engineers: St. Joseph, MI, USA, 2012.
27. Jindal, V.K.; Siebenmorgen, T.J. Effects of oven drying temperature and drying time on rough rice moisture content determination. *Am. Soc. Agric. Biol. Eng.* **1987**, *30*, 1185–1192. [CrossRef]
28. Cheng, X.; Cheng, J.; Huang, X.; Lai, Y.; Wang, L.; Du, W.; Wang, Z.; Zhang, H. Dynamic quantitative trait loci analysis of seed reserve utilization during three germination stages in rice. *PLoS ONE* **2013**, *8*, e80002. [CrossRef] [PubMed]
29. Soltani, A.; Gholipoor, M.; Zeinali, E. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environ. Exp. Bot.* **2006**, *55*, 195–200. [CrossRef]
30. Lenth, R.; Love, J.; Herve, M. Population marginal means in the linear model: An alternative to least squares means. *Am. Stat.* **2018**, *34*, 216–221. [CrossRef]
31. Baker, C.J.; Roberts, D.P.; Mock, N.M.; Blount, V.L. A novel open-system technique to monitor real-time oxygen consumption during early phases of seed germination. *Seed Sci. Res.* **2004**, *14*, 17–26. [CrossRef]
32. Tang, Y.; Gao, F.; Guo, S.; Li, F. Effects of hypobaria and hypoxia on seed germination of six plant species. *Life Sci. Sp. Res.* **2014**, *3*, 24–31. [CrossRef]
33. Graham, I.A. Seed storage oil mobilization. *Annu. Rev. Plant Biol.* **2008**, *59*, 115–142. [CrossRef]
34. Simon, E.W.; Meany, A. Utilization of reserves in germinating phaseolus seeds. *Plant Physiol.* **1965**, *40*, 1136–1139. [CrossRef]
35. Gonzàlez-Meler, M.A.; Ribas-Carbo, M.; Giles, L.; Siedow, J.N.; Gonzàlez-Meler, M.A.; Ribas-Carbo, M.; Giles, L.; Siedow, J.N. The Effect of Growth and Measurement Temperature on the Activity of the Alternative Respiratory Pathway1. *Plant Physiol.* **1999**, *120*, 765–772. [CrossRef] [PubMed]
36. Lin, T.-Y.; Markhart, A.H. Temperature Effects on Mitochondrial Respiration in *Phaseolus acutifolius* A. Gray and *Phaseolus vulgaris* L. *Plant Physiol.* **1990**, *94*, 54–58. [CrossRef] [PubMed]
37. Bello, P.; Bradford, K.J. Single-seed oxygen consumption measurements and population-based threshold models link respiration and germination rates under diverse conditions. *Seed Sci. Res.* **2016**, *26*, 199–221. [CrossRef]
38. Kuznetsov, O.A.; Hasenstein, K.H. Oxygen requirement of germinating flax seeds. *Adv. Sp. Res.* **2003**, *31*, 2211–2214. [CrossRef] [PubMed]

39. Misra, N.M.; Kar, R.K. Regulation of storage protein degradation in cotyledons of germinating cowpea. *Plant Physiol.* **1990**, *33*, 333–339.
40. Yewle, N.R.; Stroshine, R.L.; Ambrose, R.K.; Baributsa, D. Short-term hermetic storage of wet maize and its effect on quality. *Foods* **2023**, *12*, 891. [CrossRef]
41. Villers, P.; Navarro, S.; DeBruin, T. Development of hermetic storage technology in sealed flexible storage structures. In Proceedings of the 8th International Conference on Controlled Atmosphere and Fumigation in Stored Products, Chengdu, China, 21–26 September 2008; Daolin, G., Navarro, S., Jian, Y., Cheng, T., Zuxun, J., Yue, L., Haipeng, W., Eds.; Sichuan Publishing Group: Sichuan, China, 2008; pp. 21–26.
42. Sahu, P.K.; Tilgam, J.; Mishra, S.; Hamid, S.; Gupta, A.; Jayalakshmi, K.; Verma, S.K.; Kharwar, R.N. Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. *J. Basic Microbiol.* **2022**, *62*, 647–668. [CrossRef]
43. Franco-Duarte, R.; Černáková, L.; Kadam, S.; Kaushik, K.S.; Salehi, B.; Bevilacqua, A.; Corbo, M.R.; Antolak, H.; Dybka-Stępień, K.; Leszczewicz, M.; et al. Advances in Chemical and Biological Methods to Identify Microorganisms—From Past to Present. *Microorganisms* **2019**, *7*, 130. [CrossRef]
44. Nell, M.; Mammerler, R.; Steinkellner, S. Oxygen consumption-based evaluation of fungal activity. *Mycol. Res.* **2006**, *110*, 760–764. [CrossRef]