agriculture

MDPI

Article

Effect of Anaerobic Soil Disinfestation on Tuber Vitality of Yellow Nutsedge (*Cyperus esculentus*)

Jeroen Feys [1,*], Dirk Reheul [1], Wolf De Smet [1], Shana Clercx [2], Sander Palmans [2], Gert Van de Ven [3] and Benny De Cauwer [1]

[1] Weed Science Unit, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, 9000 Gent, Belgium; benny.decauwer@ugent.be (B.D.C.)
[2] Educational Research Center (PVL), 3950 Bocholt, Belgium
[3] Hooibeekhoeve, 2440 Geel, Belgium
* Correspondence: jdfeys.feys@ugent.be

Abstract: *Cyperus esculentus* is considered the sixteenth worst weed in the world. The weed causes huge losses in arable crops. Current control strategies are based on combinations of chemical and mechanical methods, repeated over years, and aim to deplete the belowground bud bank. However, this is a slow process. Anaerobic soil disinfestation (ASD) using readily decomposable carbon sources may be a promising innovative method to quickly deplete the bud bank. This study investigated the effect of ASD with fresh grass clippings (dosage of 80 tonnes ha^{-1}) differing in C:N ratio and Herbie® (consists of organic by-products from the food processing industry, dosage of 25 tonnes ha^{-1}) on the vitality of small and large *C. esculentus* tubers buried at three depths (5, 15, and 30 cm) into two soils differing in soil type and soil moisture content. Their effects were compared with the effect of chemical soil disinfestation (CSD) with metam-sodium (153 kg ha^{-1}). ASD with Herbie® showed at least equal performance compared with CSD with metam-sodium, with reductions in tuber vitality up to 97.5%. The performance of ASD with grass clippings was less consistent across soils and was affected by the C:N ratio of the grass. Both ASD and CSD showed the highest performance in moist, sandy soil and on small tubers. ASD is an effective and promising method to quickly deplete the *C. esculentus* bud bank, provided that the soil is sandy and moist, the carbon source has a C:N ratio of about 10, and the incorporation depth is at least 25 cm. To foster the implementation of ASD, future research should evaluate its performance consistency across environments and years.

Keywords: mother tuber; biological weed control; soil disinfestation; fermentation; soil moisture content; soil texture

Citation: Feys, J.; Reheul, D.; De Smet, W.; Clercx, S.; Palmans, S.; Van de Ven, G.; De Cauwer, B. Effect of Anaerobic Soil Disinfestation on Tuber Vitality of Yellow Nutsedge (*Cyperus esculentus*). *Agriculture* **2023**, *13*, 1547. https://doi.org/10.3390/agriculture13081547

Academic Editor: Yuan Li

Received: 30 June 2023
Revised: 28 July 2023
Accepted: 31 July 2023
Published: 2 August 2023

1. Introduction

Cyperus esculentus L. (yellow nutsedge) is considered the sixteenth worst weed in the world [1]. The weed is very hard to control and can lead to huge losses in arable crops. For example, the weed can lead to a yield loss of 60% in sugar beets (*Beta vulgaris* L.) and 40% in potatoes (*Solanum tuberosum* L.) [2]. High infestation levels (80–100% of the field covered with shoots) can lead to yield losses of 86%, 90%, and 93% in leek (*Allium porrum* L.), onions (*Allium cepa* L.), and Brussels sprouts (*Brassica oleracea* var. *gemmifera* (de Candolle) Zenker), respectively [3]. In maize (*Zea mays* L.), 8% yield reduction can be expected for every 100 shoots per square meter [4]. Reproduction occurs mainly via axillary buds on tubers and basal bulbs that are both formed subterranean at the tip rhizomes [5]. When the soil temperature is over 12 °C, a mother tuber starts to germinate, and the primary shoot arises [6]. Simultaneously, a subterranean basal bulb is formed. This basal bulb initiates the formation of rhizomes, which generate new basal bulbs and shoots. Later in the growing season, when daylength shortens, the rhizomes initiated from basal bulbs progressively generate daughter tubers instead of shoots. Shoot and tuber production can be very prolific.

Depending on clonal origin, a single mother tuber planted in a 10 L container can give rise to 29 to 91 shoots and 187 to 638 daughter tubers in one growing season [7]. The most persistent mother tubers may remain germinative for 10 years [8]. *C. esculentus* also produces seeds, but most of these seeds are not able to germinate in the field [9].

In Belgium, over 50,000 hectares of cropland are infected with *C. esculentus* (Feys, pers. comm.). Currently, control strategies are mostly based on herbicide applications in maize, as maize is the only crop in which selective herbicides with activity against *C. esculentus* are approved. Maize is also a good competitor once its canopy is closed [10]. However, single control measures are not sufficient to obtain complete control of *C. esculentus* [11,12]. Moreover, the European Commission plans to halve the overall use of pesticides (and associated risks related to human health and the environment) by 2030, as stated in the EU Green Deal framework. Thus, there is a need for alternative curative control strategies. Frequent soil tillage may complement chemical control strategies but entrain a high risk of tuber dispersal [12].

Anaerobic soil disinfestation is a possible control method for reducing the *C. esculentus* soil tuber bank. Anaerobic soil disinfestation (ASD) is considered an alternative to chemical soil fumigation (CSF) [13]. Chemical soil fumigation with metam-sodium provides good control of *C. esculentus* tubers [14], and metam-sodium is actually the sole synthetic fumigant legally approved for CSD in Belgium. In a three-year field experiment with polyethylene covered seedbeds, Johnson and Mullinix [15] found that metam-sodium at a dose of 190.74 kg ha^{-1} led to an average *C. esculentus* control of 84%. But limitations on the use of chemical soil fumigants are becoming more apparent due to environmental and safety concerns [16], so there is a need for alternatives like ASD. According to Shrestha et al. [17], ASD may affect the vitality of weed propagules. In ASD, readily decomposable carbon sources are incorporated into the soil. Thereafter, the soil is irrigated to saturation and covered with an impermeable plastic tarp for several weeks to create anaerobic soil conditions [18–20]. After covering the soil, the growth of aerobic microorganisms is stimulated by the organic amendment, which leads to a fast decline in oxygen concentration in the soil. Then, the facultative and obligate anaerobic microorganisms become dominant over the aerobic microorganisms [13]. Meanwhile, strong, reducing soil conditions are created [19]. Under these conditions, fatty acids like (iso)butyric acid, maleic acid, (iso)valeric acid, acetic acid, lactic acid, citric acid, and propionic acid, as well as volatile organic compounds such as ammonia, hydrogen sulfide, and nitrous oxide, are produced. The production of fatty acids lead to increased soil acidity [20,21]. Heavy metal ions, such as Fe^{2+}, Al^{2+}, and Mn^{2+}, will accumulate in the soil. According to Pakeman et al. [22], aluminum or other metals might decrease seed longevity at low pH. These alterations in the soil might have a negative impact on the growth of future crops. Muramoto et al. [23] evaluated the effect of ASD with only rice bran (20 tonnes ha^{-1}) and a combination of rice bran (6.7 tonnes ha^{-1}) with mustard seed meal (4.5 tonnes ha^{-1}) on the growth of strawberry (*Fragaria* × *ananassa* Duch.) plants. In the beds treated with rice bran and mustard seed meal, some salt damage was observed. Moreover, the treatment with only rice bran lowered the soil pH one unit more compared to the untreated control, which might be a problem for future crops. On the other hand, Vecchia et al. [24] observed that ASD with molasses or different cover crops as a carbon source did not cause any plant stunting or phytotoxicity in lettuce (*Lactuca sativa* L.).

Strauss and Kluepfel [25] stated that the efficacy of ASD is dependent on three parameters: the carbon source used, the tarp type, and the soil temperature. Shrestha et al. [26] performed a pot experiment to evaluate the effect of anaerobic soil disinfestation with various carbon sources differing in C:N ratio on *C. esculentus* tuber germination at different depths. At a depth of 15 cm, tuber germination was 60% lower after incorporation of wheat bran with a C:N ratio of 13.3 than after incorporation of dry molasses with a C:N ratio of 29.2. However, no such effect was observed for tubers buried at a depth of 5 cm. The use of a suitable plastic tarp is very important, as anaerobic soil conditions must be created. This has been confirmed by Lamers et al. [27], showing better control of *Verticillium dahlia* and *Pratylenchus penetrans* when soil was covered with a three-layer film

of polyethylene and polyamide (so-called VIF, or Virtually Impermeable Film, with very high oxygen impermeability) instead of a standard ensilage polyethylene sheet. After ASD with a standard ensilage foil, 0.5 microsclerotia per gram of soil were detected. When ASD with a VIF foil was performed, the contamination decreased to barely detectable levels. Finally, Shrestha et al. [20] found in a meta-analysis that the reduction of soilborne pathogens (e.g., *Sclerotinia*, *Rhizoctonia*, and *Fusarium*) by ASD was on average 10% higher at high soil temperatures (>35 °C) than at low (<16 °C) or moderate (16–35 °C) soil temperatures.

ASD was originally developed to suppress soilborne plant pathogens; however, studies indicate that ASD might be used as a weed management tool, especially in zones where herbicides are not permitted. Multiple authors investigated the effect of ASD on *C. esculentus* vitality [18]. Liu et al. [28] found that ASD with different carbon sources (buckwheat, cowpea, velvet bean, paper mulch, and rice bran) led to a significant reduction in *C. esculentus* tuber sprouting compared to an untreated control. The reduction in germination rate varied from 45.0 to 60.0%, depending on the carbon source. Shrestha et al. [17] found that the burial depth of the *C. esculentus* tubers affects the effectiveness of an ASD treatment. At a depth of 15 cm, an ASD treatment with dry molasses-based or wheat bran-based amendments (both at a dose of approximately 10 g per kg of soil) led to a significant reduction in tuber sprouting. However, this significant reduction was not observed at a depth of 5 cm. This reduced effectiveness of ASD at shallower depths might be explained by oxygen exchange and soil moisture evaporative losses at the soil-atmosphere boundary.

Several carbon sources can be used for ASD. In Belgium, there is an abundant supply of fresh grass, as approximately 35% of the agricultural area is covered by permanent grassland [29]. ASD with freshly chopped grass is already applied to open-field vegetables [30]. Herbie® is another interesting carbon source that is commercially available for ASD purposes. Herbie® has been developed in the Netherlands and is a particulate or liquid organic product that comprises one or more of the various types of protein-containing agricultural by-products (e.g., extracted rapeseed meal, extracted soya bean meal, gluten, steamed potato peelings, protamylasse, or thick potato sap) and contains at least 10 wt-% of proteins and/or at most 90 wt-% of rapidly degradable carbohydrates and/or lipids all on a dry-matter basis, totaling 100 wt-% [31]. Unlike chopped grass (C:N ratio between 12 and 25), it has a constant C:N ratio of approximately 10.4 [32,33]. In a closed bucket experiment, Hoek et al. [34] investigated the effect of ASD with fresh grass and Herbie® on the sprouting capacity of *C. esculentus* tubers originating from two clonal populations. ASD with grass (15 g L^{-1} soil) or Herbie® (26.6 g liquid product L^{-1} soil corresponding to 0.29 g N L^{-1} soil) led to a significant reduction (up to 86.8%) in tuber sprouting capacity compared to the untreated control (fridge stored tubers). No significant differences in tuber sprouting capacity were observed between ASD with grass and Herbie® (sprouting capacities of 0.2 and 0.5%, respectively). Moreover, no interclonal variability in sensitivity to ASD, was found. However, the authors did not verify whether the clones were genetically different. Possibly, the genetic background of the clone may affect the sensitivity to ASD as shown for herbicide sensitivity by De Cauwer et al. [7]. The results of the experiment by Hoek et al. [34] indicate that ASD with fresh grass and Herbie® is a promising tool for controlling *C. esculentus* tubers. However, the effects need to be confirmed in field experiments under different pedohydrological conditions.

In our study, the effect of ASD with fresh grass and Herbie® on *C. esculentus* tuber vitality was investigated in field experiments. Their effects were compared with thoseof chemical soil disinfestation (CSD) with metam-sodium. Moreover, the potential effects of the parameter's tuber burial depth, genetic clone, soil texture, and soil moisture content were evaluated. The following hypotheses were formulated: (H1) ASD with fresh grass clippings and Herbie® significantly affects *C. esculentus* tuber vitality, regardless of the burial depth of tubers, and is a good alternative for CSD with metam-sodium; (H2) the efficacy of ASD with fresh grass clippings and Herbie® is affected by the genetic diversity of clones; (H3) the efficacy of ASD with fresh grass clippings and Herbie® or CSD with metam-sodium depends on soil properties. To address these hypotheses, the following

research questions were formulated: (i) What is the impact of ASD with freshly chopped grass and Herbie® and CSD with metam-sodium on the vitality of *C. esculentus* tubers buried at different depths? (ii) To what extent does the clonal genetic background affect the efficacy of grass- and Herbie®-based ASD? (iii) To what extent do soil texture and soil moisture content affect the efficacy of grass- and Herbie®-based ASD?

2. Materials and Methods

2.1. Experimental Sites

During the summer of 2022, the efficacy of ASD was evaluated by performing two field experiments at Bree and Mol, both located in the northeastern part of Belgium. The distance between the two locations is approximately 41 km. Table 1 gives more information about the soils at Bree and Mol. During the last few years, silage maize was grown at both locations.

Table 1. The characteristics of the soils at Bree (sandy loam) and Mol (sand).

Location	Bree	Mol
Soil texture	Sandy loam	Sand
Content of clay, loam, sand (respectively)	4.0%, 24.5%, 69.5%	>90% sand
Organic carbon content (%)	1.70	1.15
pH_{KCl}	5.3	5.7
Bulk density 0–30 cm (kg m^{-3})	1430	1286
Moisture content 0–30 cm (gravimetric, %)	16.6	24.7
Moisture content 0–30 cm (volumetric, %)	23.7	31.8
Field capacity (m^3 m^{-3}) [1]	0.14	0.17
Permanent wilting point (m^3 m^{-3}) [1]	0.05	0.025–0.09
Porosity (m^3 m^{-3}) [1]	0.45	0.43
Drainage class [2]	d	e

[1] These values were estimated using the literature data [35,36]. [2] Drainage classes as derived from the digital soil map of Belgium: d = imperfectly drained; e = poorly drained [37].

2.2. Experimental Set-Up

At both locations, a randomized block design with four replicates were used. All combinations of four treatments (untreated tarped control, ASD with grass clippings, ASD with Herbie®, and CSD metam-sodium), three burial depths (5, 15, and 30 cm), and two genetically different clones (clones producing heavy and light tubers) were evaluated. Detailed information about these experimental factors is given in Section 2.2.1. An experimental unit consisted of a water- and gas-permeable nylon bag filled with 30 tubers. These bags measured 30 cm by 25 cm and were handmade using woven Suzuki fruit fly net (Duranet, Oostende, Belgium) with a mesh size of 0.97 mm × 0.83 mm.

2.2.1. Experimental Factors

Four treatments were evaluated: grass, Herbie®, metam-sodium and an untreated tarped control. The freshly chopped perennial ryegrass (*Lolium perenne* L.) and Herbie® (particulate product) were incorporated in the soil at a dose of 80 and 25 tonnes ha^{-1}, respectively (see Section 2.2.2). Dose rates used were recommended by agronomic advisors (grass clippings) and by Soilwise (the producer/supplier of Herbie®). For Herbie®, this dose is close to the optimal dose of 2 g of crude protein per liter of soil found in pot experiments conducted by Hoek et al. [34]. At both locations, the grass was grown next to the field trial and was mown (at 9 a.m.) and chopped (at 18 p.m.) the day before the start of the experiment. Herbie® was purchased at Soilwise® (Wageningen, The Netherlands). Metam-sodium based CSD was performed with Nemasol (510 g L^{-1} metam-sodium, SL, Taminco, Ghent, Belgium) at the maximum field dose of 300 L ha^{-1}. The characteristics of the fresh grass clippings (at locations Bree and Mol), Herbie®, and Nemasol are given in Table 2. Relative to grass clippings from location Bree, grass clippings from location Mol had a lower C:N ratio and a higher N content. The calculated applied amounts of carbon (C), sulfur (S), and nitrogen (N) corresponding with the grass and Herbie® dosages

are given in Table 3. Lastly, there was also an untreated, tarped control. Tarping of the control plots was performed to prevent the sprouting of buried mother tubers. Massive sprouting would deplete the mother tubers and hence negatively affect their vitality. During the experimental periods, 120 tubers (4 repeats of 30 tubers) of the same genetic clones as in the field experiments were stored in the fridge (5 °C). After the experiment, the vitality of these fridge stored tubers (which was 90.1 ± 2.74% and 90.5 ± 3.05% at Bree and Mol, respectively), was compared with the vitality of the tubers in the tarped control plots. Thereby, the effect of tarping on tuber vitality could be assessed.

Table 2. The dose, dry matter (DM) content (%), C:N ratio, and total N-, S-, and C-content (expressed in g per kg dry matter) for each disinfestation material.

Disinfestation Material	Dose	DM Content [1] (%)	S-Content [2] per kg DM (g)	C-Content [3] per kg DM (g)	N-Content [4] per kg DM (g)	C:N Ratio
Grass (Bree)	80 tonnes ha^{-1}	35.8	2.38	408	23.2	17.6
Grass (Mol)	80 tonnes ha^{-1}	20.2	2.20	400	26.3	15.2
Herbie®	25 tonnes ha^{-1}	92.0	2.62	439	42.3	10.4
Metam-sodium [5]	153 kg a.i. ha^{-1}	/	/	/		/

[1] Dry matter content was obtained after 16 h of drying at 75 °C. The dry matter content of the grass clippings was measured just before incorporation into the soil. [2] S-content was obtained using the CMA 2/IV/19 and ICP-OES standards [38]. [3] C-content was obtained using the ISO 10694 standard [39]. [4] N-content was obtained using the Dumas method, according to the ISO 16634-1 standard [40]. [5] Formulated product: Nemasol (510 g L^{-1} metam-sodium, SL, Taminco)/Irrelevant.

Table 3. Nitrogen (kg ha^{-1}), sulfur (kg ha^{-1}), and carbon (kg ha^{-1}) supplies added to the soil by incorporation of grass clippings and Herbie® at a dose rate of 80 tonnes ha^{-1} and 25 tonnes ha^{-1}, respectively.

Disinfestation Material	N Dose (kg ha^{-1})	S Dose (kg ha^{-1})	C Dose (kg ha^{-1})
Grass (Bree)	663	68	10,098
Grass (Mol)	424	36	6461
Herbie®	974	60	11,672

Tubers were buried at 3 depths: 5 cm, 15 cm, and 30 cm. Tubers at a depth of 30 cm were outside the incorporation zone of grass clippings, Herbie®, or metam-sodium.

Two genetically different *C. esculentus* clones were included in the experiments, namely clone 'Meulebeke', which produces relatively heavy tubers, and clone 'Bree', which produces relatively light tubers. The clones were named after the place in Belgium where they were originally sampled. De Ryck et al. [41] found that these clones belong to two genetically different clusters based on an AFLP analysis. The tubers used in the experiments were produced and harvested in 2021 and kept in the fridge at 5 °C until usage.

2.2.2. Soil Disinfestation Process

At both locations, the trial field consisted of 16 experimental plots (4 treatments × 4 replicates), each measuring 5 m × 3.80 m at location Bree and 10 m × 6 m at location Mol. In each plot, 6 nylon bags (3 burial depths × 2 clones) were buried.

The experiments were set-up on 14 June (Bree) and 22 June (Mol). The soil moisture content of the topsoil (0–30 cm) at the time of set-up was 16.6% at Bree and 24.7% at Mol. After demarcating the plots, the necessary amendments of fresh grass clippings and Herbie® were calculated per plot, weighed, and evenly distributed across each plot (Tables 2 and 3). Immediately after spreading, amendments were incorporated in the soil to a depth of 25 cm using a PTO-driven spader (Imants 48 SX, Imants BV, Reusel, The Netherlands) with a working width of 3 m. Thereafter, the soil was rolled. The treatment with metam-sodium was performed by a specialized contractor with the phytolicence required for CSD. Hereby, the metam-sodium injection and incorporation (to a depth of 20 cm) and the rolling of the soil were performed consecutively in 1 working pass. After

incorporation of all soil disinfestation materials, nylon bags filled with *C. esculentus* tubers were buried in the central zone of each plot (i.e., at least 1.5 m away from the plot edge) to avoid edge effects. Along plot edges, anoxic conditions required for optimal efficacy of ASD may not be reached as a result of oxygen diffusion from untarped zones into the treated zones. Burying was performed by digging a small trench of 5, 15, or 30 cm depth (checked by a ruler), placing a nylon bag with *C. esculentus* tubers in a horizontal position on the bottom of the trench, refilling the trench layer by layer with the dugout earth, and slightly compressing the filled trench with a small roller. For safety precautions, a gas mask and a disposable overall were used to bury the nylon bags in metam-sodium plots.

After burying the tubers, all experimental plots (including the untreated control) were tarped for 6 weeks. The treatment duration of 6 weeks was chosen as ASD generally takes 3–6 weeks [25]. For tarping, a transparent virtually impermeable film (VIF) was used. This film is highly impermeable to gases but light-permeable. The film is 35 µm thick and has an oxygen transmission rate of 40 cm^3 m^{-2} day^{-1}. The strength of the film can be described by the dart drop and the tensile strength at break. These are 180 g and 35 MPa, respectively.

At location Bree, tarping was performed by a specialized contractor who constructed a machine for automatic tarping (Figure 1). At both ends of the working width, the machine digs out a small gully in which the foil is laid. Thereafter, the gullies are filled up again to belay the foil. The machine has a working width of 3.8 m, which is also the width of the plots at location Bree. At location Mol, the experimental plots were manually covered with VIF tarp as the plot width (6 m) exceeded the working width of the tarping machine. To prevent bird damage to the VIF tarp at the bird-rich location Bree and to maintain anoxic conditions, a standard black-on-white coextruded silage foil (not airtight) was placed over all experimental plots with the black side upwards. Birds tend to prick holes in the transparent VIF tarp in their attempt to reach the earthworms sticking to the underside of the tarp.

Figure 1. Machine for automatic tarping.

After 6 weeks, on 26 July (Bree) and 3 August (Mol), nylon bags were exhumed and stored at 5 °C awaiting tuber vitality assessment. The climatic conditions during these experimental periods are given in Figure 2. Also, the oxygen concentration in the soil pores and the gas composition of the air (under the foil) were measured during the experiments.

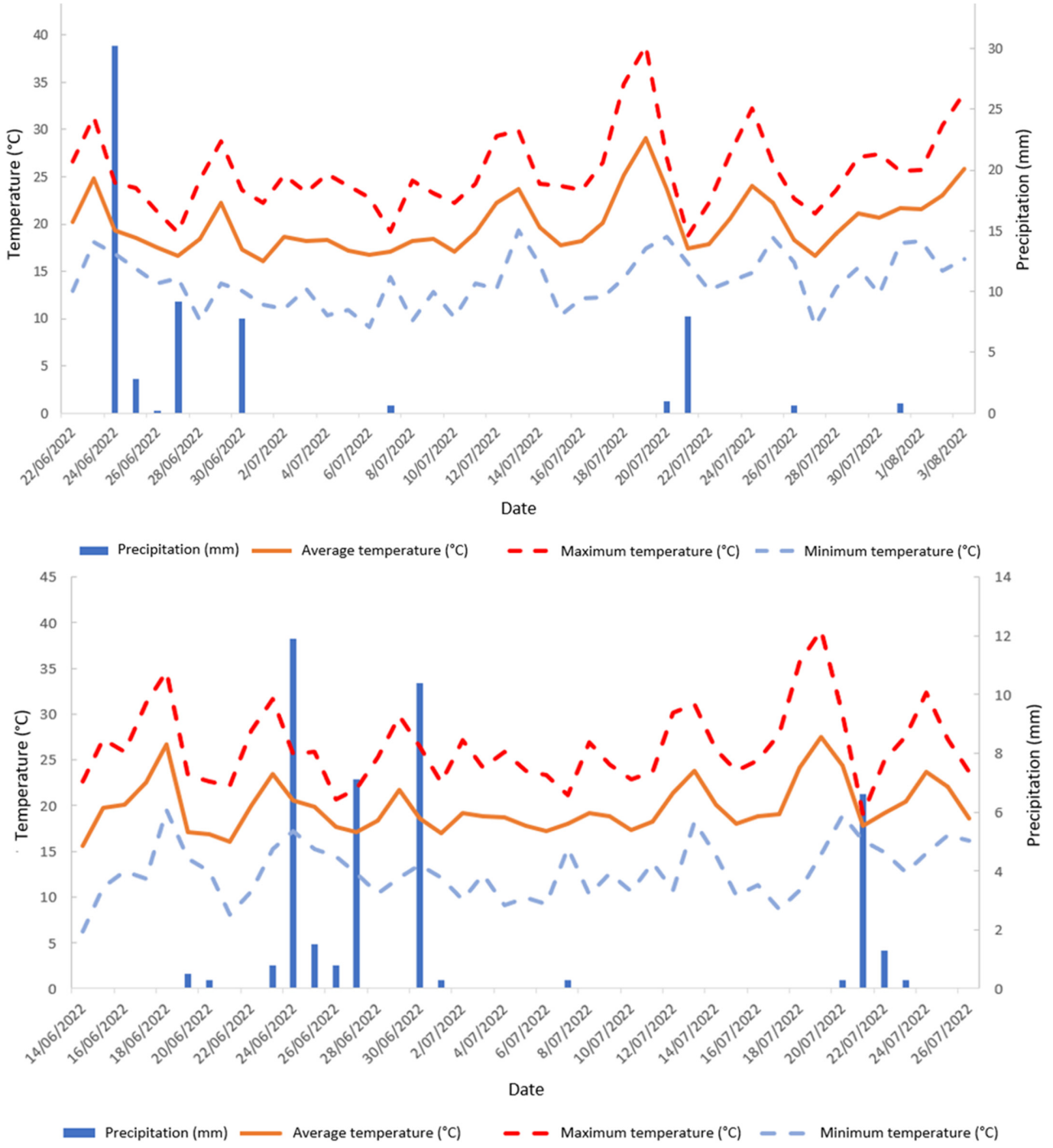

Figure 2. Minimum, average, and maximum temperatures (°C) and precipitation (mm) during the experimental periods. (**Top**): location Bree (14 June–27 July). (**Bottom**): location Mol (22 June–3 August).

2.3. Measurements

2.3.1. Gas Composition

To characterize and determine the intensity of fermentation processes in tarped soil, the soil oxygen concentration and the gas composition of the headspace under the VIF foil were periodically measured during the 6-week incubation period of ASD and CSD. Measurements were performed on 20 June, 29 June, 7 July, 21 July and 26 July at Bree,

and on 29 June, 5 July, 21 July, and 3 August at Mol. The soil oxygen concentration (volume %), was determined at a depth of 20 cm using a soil oxygen analysis system (Royal Eijkelkamp, Giesbeek, The Netherlands). The system consists of a piercing probe and an oxygen concentration meter with an electrochemical cell [42]. Gas composition in the headspace under the tarp was characterized by measuring the O_2 (volume %), NH_3 (ppm), and H_2S (ppm) concentrations in the headspace using a Honeywell BW Ultra gas detector (Honeywell Analytics, Calgary, AB, Canada). These gases are produced during fermentation and are toxic to living organisms [43].

2.3.2. Tuber Vitality

Tuber vitality was assessed by performing a germination test followed by a tetrazolium test. Six weeks after soil tarping, on 26 July (Bree) and 3 August (Mol), the nylon bags each containing 30 *C. esculentus* tubers were carefully exhumed and stored in the fridge at 5 °C. The next day, exhumed tubers and reference tubers stored for 6-weeks at 5 °C were transferred to a Copenhagen germination table (regime of 16 h light at 24 °C and 8 h dark at 18 °C) to determine the number of sprouted tubers. Heretofore, tubers were placed on top of moistened Rotilabo® filters (diameter 90 mm, type 112A, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), one per experimental unit of 30 tubers. After 10 days of incubation, sprouted tubers were counted, and the non-sprouted tubers were subjected to a tetrazolium test to evaluate their vitality. Heretofore, the tubers were cut longitudinally into two halves. One half of the cut tuber was thrown away; the other half was laid in a petri dish (with the cutting surface downward). Selected tuber halves belonging to the same experimental unit were laid down in the same petri dish with a Rotilabo® filter (diameter 90 mm) moistened with 4 mL of a tetrazolium solution (10 g 2,3,5-trifenyl-2H-tetrazolium-chloride [TZ] L^{-1}) and incubated for 24 h at 23 °C in complete darkness. If TZ makes contact with metabolically active *C. esculentus* tissue, TZ will convert to formazan by a hydrogenation reaction [44]. This reaction can be observed visually, as TZ and formazan are colorless and carmine red, respectively. Thus, the more metabolic activity in a certain tuber, the more the cutting surface will be colored carmine red after a tetrazolium test. Figure 3 gives an illustration of this test. In our experiments, the amount of coloring was scored on a 0-to-10 scale (0 = no coloring, 10 = very clear, complete coloring). Tubers with a score of 5 or higher were considered dormant (metabolically active) tubers. For each experimental unit, tuber vitality (%) was calculated by dividing the number of living tubers (i.e., the sum of the numbers of sprouted and dormant tubers) by the total number of tubers (=30).

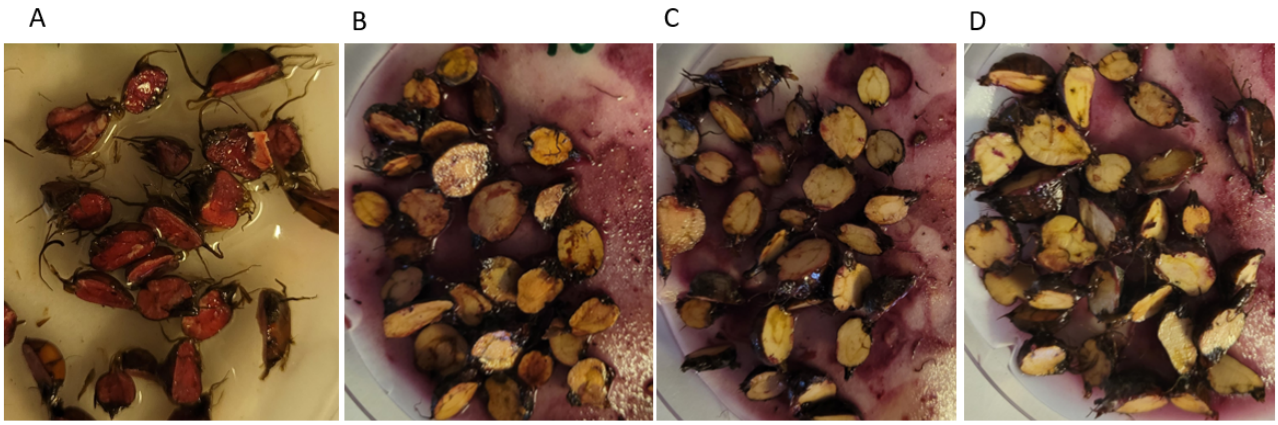

Figure 3. Illustration of the tetrazolium test. Tetrazolium-chloride-treated tuber halves of untreated fridge-stored tubers (**A**), tubers from ASD-treated plots using grass clippings (**B**) or Herbie® granules (**C**), and tubers from metam-sodium-treated plots (**D**) (location Mol, clone with heavy tubers, burial depth of 15 cm). Vital tubers have a red-colored cutting surface.

2.4. Statistical Data Analysis

All data were analyzed in Rstudio, version 4.1.3 [45], using parametric or non-parametric tests run at the 5% significance level. Gas measurement data were analyzed using one-way anova tests. The assumptions of normality and homoscedasticity were checked with a QQ-plot and the Levene Test, respectively. If these assumptions were met, the TukeyHSD test was performed to detect significant differences between factor levels. Tuber vitality data were analyzed using three-way-anovas to detect significant main and interaction effects of the factor's treatment, tuber burial depth, and clone. Then, reduced models were constructed. Model assumptions were checked as described above. As these assumptions were met, the Tukey HSD test was used to search for significant differences between factor levels (for all significant main and interaction factors). To evaluate the effect of tarping on tuber vitalities, the vitalities of tubers exhumed from the field plots were compared with the vitalities of fridge- stored tubers using *t*-tests. Hereby, the assumption of homoscedasticity was checked. If this assumption was met, the two-sample *t*-test was used. If not, Welch's *t*-test was used.

3. Results

3.1. Location Bree

3.1.1. Gas Measurements (Figure 4)

Soil Oxygen

After six days of incubation, the metam-sodium plots showed significantly higher soil oxygen concentrations compared to the other treatments (4.0% vs. less than 1.0%). After 15 days of incubation, the soil oxygen concentration in the metam-sodium plots declined to similar levels as in the plots of the other treatments (between 0.0% and 1.0%). After 37 days, the soil oxygen concentration in metam-sodium and control plots was slightly increased (but not significantly) to a level of1.4% and 1.2%, respectively. In the grass and Herbie® plots, no soil oxygen was found from 15 days to 37 days of incubation.

Gas Composition in the Headspace under the VIF

Between 6 and 23 days of incubation, the oxygen concentration in the headspace was significantly higher in the control plots than in the grass plots (9.2% vs. 1.2%, 4.3% vs. 1.7%, and 5.1% and 0.9% at days 6, 15, and 23, respectively). After 23 days, the metam-sodium plots also showed a significantly higher oxygen concentration than the grass plots (5.1% vs. 0.9%). However, after 37 days of incubation, no significant differences in oxygen concentration were found among treatments. The oxygen concentration varied between 2.0% (Herbie® plots) and 5.1% (control plots).

After 6 days of incubation, the hydrogen sulfide concentration was significantly higher in the Herbie® plots than in the metam-sodium or grass plots (2.1 ppm, 0.2 ppm, and 0.0 ppm, respectively). Between day 6 and day 15, hydrogen sulfide concentration has been increasing rapidly in metam-sodium and grass plots (2.2 ppm and 2.1 ppm, respectively), whereas it has been decreasing in Herbie® and control plots (0.0 ppm and 0.15 ppm, respectively). After 23 days of incubation, a decline in hydrogen sulfide concentration was observed in metam-sodium plots. Hereby, the hydrogen sulfide concentration was significantly higher in the grass plots than in the other plots (2.1 ppm vs. 0.2 ppm or less). After 37 days of incubation, hydrogen sulfide concentrations varied between 0.0 ppm and 0.2 ppm and revealed no significant differences among treatments.

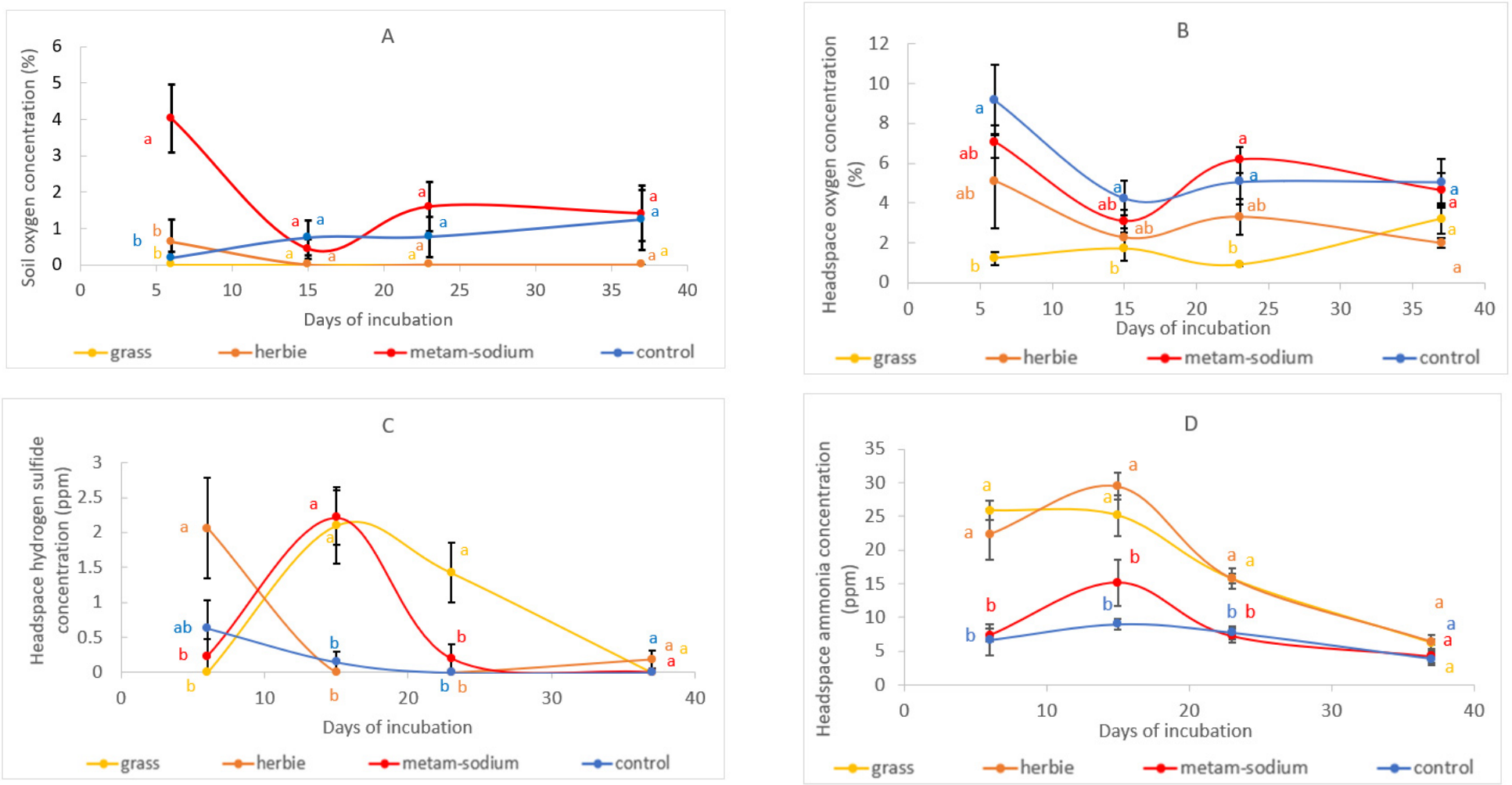

Figure 4. Evolution of gas contents over time for four soil disinfestation treatments during incubation at Bree. (**A**): Soil oxygen concentration (%) at 20 cm depth. (**B**): Oxygen concentration (%) in the headspace of the foil. (**C**): Hydrogen sulfide concentration (ppm) in the headspace of the foil. (**D**): Ammonia concentration (ppm) in the headspace of the foil. Means without a common letter within the same measurement time are significantly different ($p < 0.05$); comparison of means within measurement time only.

From days 6 to 23 of incubation, the ammonia concentration in the headspace was significantly higher in the grass and Herbie® plots than in the metam-sodium and control plots (22.4–25.9 ppm vs. 6.6–7.4 ppm, 25.1–29.5 ppm vs. 9–15.1 ppm, and 15.8 vs. 7.3–7.8 ppm at days 6, 15, and 23, respectively), despite a sharp decline in ammonia concentration in the grass and Herbie® plots between days 15 and 23 of incubation. After 37 days of incubation, these significant differences disappeared, and ammonia concentration varied between 3.9 ppm (control plots) and 6.4 ppm (Herbie® plots).

3.1.2. Tuber Vitality

The three-way anova-test revealed a significant block effect and interaction between treatment and tuber burial depth, which was further investigated (Table 4). However, tuber vitality was not significantly affected by clone. Figure 5 depicts *C. esculentus* tuber vitality for all combinations of treatment and tuber burial depth.

Table 4. Results of the three-way anova -test performed in order to evaluate the main and interaction effects of the factors clone, tuber burial depth and treatment on *C. esculentus* tuber vitality at location Bree. Significant effects are indicated with an asterisk.

Factor	Df	F-Value	*p*-Value
Block	3	3.984	0.0112 *
Clone	1	0.602	0.4407
Tuber depth	2	15.950	2.02×10^{-6} *
Treatment	3	15.975	5.47×10^{-8} *
Clone: tuber depth	2	0.012	0.9880
Clone: treatment	3	1.740	0.16683
Tuber depth: treatment	6	3.386	0.0055 *
Clone: tuber depth: treatment	6	1.206	0.3137

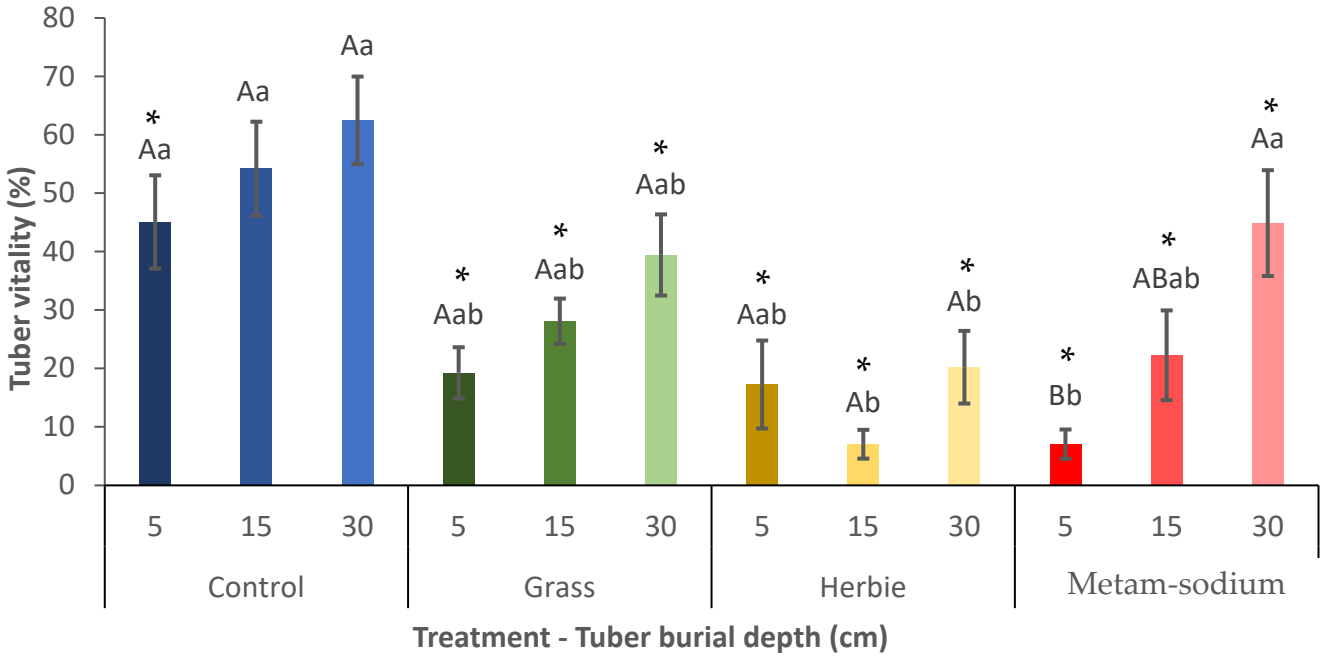

Figure 5. *C. esculentus* tuber vitality for all combinations of treatment (tarped control, grass, Herbie, or metam-sodium) and tuber burial depth (5 cm, 15 cm, or 30 cm) at location Bree. Means without a common capital letter are significantly different ($p < 0.05$), mean comparison within treatments only. Means without a common lowercase letter are significantly different ($p < 0.05$), mean comparison within tuber burial depths only. Bars with an asterisk are significantly different from the vitality of the fridge stored tubers (90.1 ± 2.74%).

Tuber vitality was not affected by burial depth irrespective of treatment except for the metam-sodium treatment. In metam-sodium plots, tuber vitality was significantly lower at a depth of 5 cm than at a depth of 30 cm (7.0 ± 2.51% vs. 44.9 ± 9.05%).

At a depth of 5 cm, reductions in tuber vitality (relative to the tarped control treatment), after 6 weeks of incubation in grass, Herbie®, and metam-sodium plots were 25.8, 27.8, and 38.0 percentage points, respectively. Except for the metam-sodium treatment all reductions were not significant. At the burial depths of 15 and 30 cm, only the Herbie® treatment led to a significant reduction in tuber vitality (reductions varying from 42.3 to 47.2 percentage points relative to the tarped control treatment). For the treatments with grass and metam-sodium, reductions in tuber vitality varied from 23.0 to 26.1 percentage points and from 17.6 to 32.0 percentage points, respectively.

At a depth of 5 cm, tubers from tarped control plots had a significantly lower tuber vitality than the tubers stored in the fridge for six weeks. The vitality of these tubers was 51.5 ± 12.36% and 90.1 ± 2.74%, respectively. At depths of 15 and 30 cm, there were no significant differences in tuber vitality between tubers from tarped control plots and fridge stored tubers. Hence, only at a depth of 5 cm did tarping significantly affected tuber vitality.

3.2. Location Mol

3.2.1. Gas Measurements (Figure 6)

Soil Oxygen

Up to day 30 of incubation, soil oxygen concentration at a depth of 20 cm was very low (<1.0%) and showed no significant differences among treatments. From day 29 to 42 soil oxygen concentration have been increasing again in all plots except for the Herbie® plots that remained anoxic. At day 42 of incubation, soil oxygen concentrations varied between 0.0% (Herbie® plots) and 5.0% (control plots) but were not significantly different from each other.

Gas Composition in the Headspace under the VIF

At day 7 of incubation, oxygen concentrations in the headspace under the foil varied between 1.4% (grass) and 2.5% (control). There were no significant differences among treatments. At day 13, oxygen concentrations were similar. At day 29 of the incubation, oxygen concentration in the metam-sodium plots increased to 4.2% but there were still no significant differences among treatments. At day 42, the oxygen concentration in the control plots increased to 8.3% which was significantly higher than the oxygen concentration measured in the headspace of the Herbie® plots (2.5%).

On day 7 of incubation, hydrogen sulfide concentration varied between 0.0 ppm (control) and 1.3 ppm (Herbie®). There were no statistical differences among treatments. From days 7 to 13 of incubation, hydrogen sulfide concentration was increased to 1.5 ppm in the grass plots, whereas it was decreased to significantly lower levels (0.0%) in the other plots. From days 13 to 29, the hydrogen sulfide concentration in the grass plots decreased again. From days 29 to 42, the hydrogen sulfide concentration stabilized and showed no significant differences among treatments.

From day 7 to day 42, incubation treatments showed no significant differences in ammonia concentration in the headspace under the VIF foil. After 7 days of incubation, ammonia concentrations varied between 17.9 ppm (metam-sodium) and 36.1 ppm (Herbie®). From days 13 to 29, ammonia concentrations have been increasing in grass and Herbie® plots to levels of 46.9 ppm and 64.8 ppm, respectively. However, the absence of statistically significant differences remained until the end of the experiment.

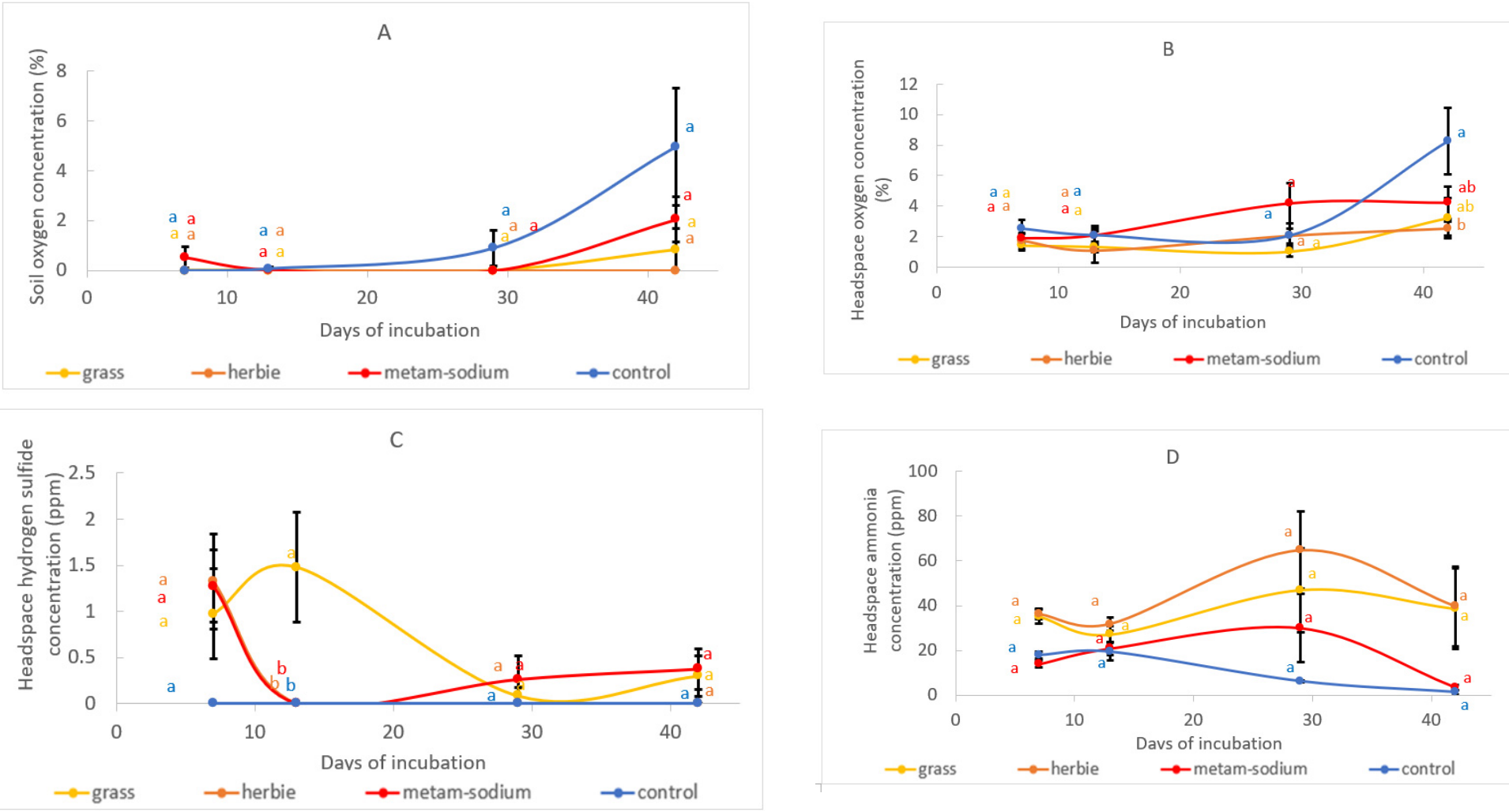

Figure 6. Gas measurements at Mol. (**A**): Soil oxygen concentration (%) at 20 cm depth. (**B**): Oxygen concentration (%) in the headspace of the foil. (**C**): Hydrogen sulfide concentration (ppm) in the headspace of the foil. (**D**): Ammonia concentration (ppm) in the headspace of the foil. All graphs: statistical comparisons of treatments only within the same point in time. Means without a common lowercase letter are significantly different ($p < 0.05$).

3.2.2. Tuber Vitality

At location Mol, the three-way anova-test indicated a significant effect of the factors treatment, clone, and block but not for tuber burial depth (Table 5). No significant interaction effects were observed. In Figure 7, *C. esculentus* tuber vitality is given for all treatments.

Table 5. Results of the three-way anova-test performed in order to evaluate the main and interaction effects of the factors clone, tuber depth, and treatment on *C. esculentus* tuber vitality at location Mol. Significant effects are indicated with an asterisk.

Factor	Df	F-Value	*p*-Value
Block	3	8.870	4.75×10^{-5} *
Clone	1	4.856	0.0310 *
Tuber depth	2	2.846	0.0649
Treatment	3	28.381	4.56×10^{-12} *
Clone: tuber depth	2	1.534	0.2229
Clone: treatment	3	0.401	0.7525
Tuber depth: treatment	6	0.710	0.6429
Clone: tuber depth: treatment	6	0.531	0.7829

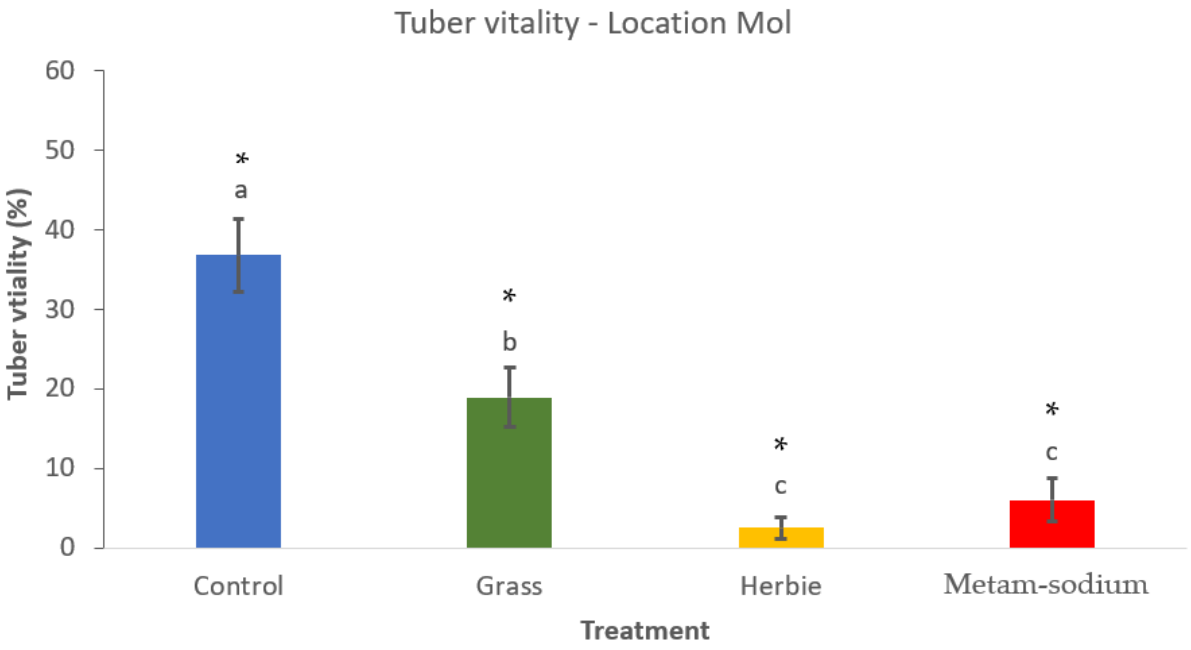

Figure 7. *C. esculentus* tuber vitality (averaged over tuber burial depths and clones) for all treatments (tarped control, grass, Herbie, and metam-sodium) at location Mol. Bars with an asterisk are significantly different from the vitality of the fridge stored tubers (90.5 ± 3.05%). Means without a common lowercase letter are significantly different ($p < 0.05$).

Compared with the tuber vitality of the tarped control treatment, the grass, Herbie® and metam-sodium treatments significantly reduced tuber vitality by 17.9, 34.3, and 30.8 percentage points, respectively. Moreover, tuber vitality was significantly lower in Herbie® and metam-sodium plots than in grass plots. Also, the factor of genetic cloning significantly affected tuber vitality. Tuber vitality, averaged over burial depths and treatments, was significantly lower for clone Bree than for clone Meulebeke (12.8 ± 2.75% vs. 19.3 ± 3.22%).

Tarping had a significant effect on tuber vitality. Averaged over burial depths, tuber vitality of the fridge- stored tubers were significantly higher than tuber vitality of tubers exhumed from tarped control (90.5 ± 3.05% vs. 36.8 ± 4.55%).

4. Discussion

Hypothesis 1 was only partly supported.

The first part of this hypothesis states that ASD with fresh grass clippings and Herbie® significantly affects *C. esculentus* tuber vitality, regardless of tuber burial depth. At Bree, this was clearly not the case. ASD with grass clippings never led to a significant reduction in tuber vitality compared to the untreated tarped control, irrespective of the tuber burial depth. On the other hand, ASD with Herbie® led to a significant reduction in tuber vitality, but only at a burial depth of 15 and 30 cm (vitality reductions of 47.2 and 42.4 percentage points, respectively). Thus, ASD with Herbie® seemed more successful at greater depths. This corresponds with the findings of Shrestha et al. [17], who executed ASD treatments with dry molasses-based and wheat bran-based amendments. At a depth of 15 cm, a significant reduction in *C. esculentus* tuber germination was observed. This effect was not observed at a depth of 5 cm. However, at location Mol, ASD with both fresh grass clippings and Herbie® led to a significant reduction in tuber vitality compared to the untreated tarped control, irrespective of the burial depth (vitality reduction of 30.8 and 34.3 percentage points averaged over burial depths, respectively).

The second part of hypothesis 1 states that ASD with fresh grass clippings or Herbie® may be a good alternative to CSD with metam-sodium. This statement is supported to a large extent, especially for ASD with Herbie®. At location Bree, ASD with Herbie® was the only treatment that significantly reduced tuber vitality of tubers buried at 15 or 30 cm, compared to the untreated tarped control. The reduction in tuber vitality at a depth of 15 and 30 cm was 47.2 and 42.3 percentage points, respectively. However, it is important to mention that the incorporation depths of the Herbie® and metam-sodium differed (25 and 20 cm, respectively). At a depth of 5 cm, CSD with metam-sodium seemed the best option to control *C. esculentus* tubers. Also, at location of Mol, ASD with Herbie® seemed like a good alternative to CSD with metam-sodium. Both treatments led to a significant reduction in tuber vitality compared to the untreated tarped control, irrespective of tuber burial depth (vitality reduction of 34.3 and 30.8 percentage points for Herbie® and metam-sodium, respectively).

As suggested in the previous paragraph, tuber vitalities obtained after ASD with Herbie® were lower than tuber vitalities obtained after ASD with fresh grass clippings. This was observed at both Bree and Mol. At Bree, the difference in tuber vitality was 2.0, 21.1, and 19.2 percentage points at a tuber burial depth of 5, 15, and 30 cm, respectively. However, these differences were not statistically significant. At Mol, averaged over tuber burial depths, tuber vitality was 16.4 percentage points lower after ASD with Herbie® compared to ASD with fresh grass clippings. This difference was statistically significant. The differential performance between ASD with Herbie® and ASD with grass can be attributed to differences in nutrient supply (as determined by carbon dose rate and chemical composition) and/or C:N ratio of the carbon source used for ASD. For example, at Mol, plots amended with 25 tonnes Herbie® per hectare received 130% more nitrogen, 67% more sulfur, and 81% more carbon than plots amended with 80 tonnes fresh grass clippings per hectare. As a result, the microorganisms dispose of a larger amount of carbon sources, which might increase the amount of methane, ethylene gases, alcohol, hydrogen sulfide, and organic acids produced [46]. Some of these substances are probably toxic to weed propagules. For example, the study of Achmon et al. [47] suggests a toxic effect of volatile fatty acids, especially acetic acid, on the seeds of *Brassica nigra* L. (black mustard) and *Solanum nigrum* L. (black nightshade). Moreover, hydrogen sulfide, produced during bacterial anaerobic respiration, would be toxic to many plant species [18] due to the inhibition of the cytochrome c oxidase enzyme in mitochondria [48]. Another possible explanation for the superior efficacy of Herbie® over grass clippings might be its lower C:N ratio (10.4 vs. 17.6 at Bree, 10.4 vs. 15.2 at Mol). Under anaerobic conditions, a low C:N ratio leads to excessive production of ammonia and volatile fatty acids [49]. Both components can adversely affect germination and plant growth [47,50]. The better performance of ASD with a carbon source with a low C:N ratio is in line with findings from laboratory ASD

experiments. Shrestha et al. [17] observed in a pot trial 34% less resprouting *C. esculentus* tubers when ASD was performed with an amendment with a C:N ratio of 10 compared to ASD with an amendment with a C:N ratio of 20. These organic amendments were created by mixing dry molasses, soybean meal, maize starch, and wheat bran. Similar effects were found in the pot experiment by Singh et al. [51]. ASD with mustard flour-molasses (C:N ratio 36) or chicken manure-molasses (C:N ratio 18) gave, respectively, 31–41% and 14–23% higher control of weeds compared to ASD with sweet potato-molasses (C:N ratio 90) and corn gluten-molasses (C:N ratio 71), respectively.

Theoretically, the gas measurements might be a good indicator of ASD effectivity. Surprisingly, the significant difference in effectivity between grass and Herbie® at Mol cannot be explained by differences in oxygen, ammonia, and hydrogen sulfide concentrations under the foil. The oxygen concentration in the soil and in the headspace under the foil, as well as the ammonia concentration in the headspace under the foil did not differ significantly among both organic amendments, irrespective of the time of gas measurement during incubation. After 13 days of incubation, the hydrogen sulfide concentration in the headspace under the foil was even significantly higher in the grass plots. However, it is very likely that the hydrogen sulfide concentration under the foil of Herbie plots peaked before the first gas measurement date (7 days after incubation). This might be explained by the very fast decomposition of Herbie® due to its relatively low NDF value of 331 g per kg of dry matter [52]. The NDF value of grass clippings is generally between 400 and 540 g per kg of dry matter, due to the higher amount of hardly degradable components such as cellulose, hemicellulose, and lignin [53].

Hypothesis 2 was partly supported. Only at Mol, the factor genetic clone significantly affected *C. esculentus* tuber vitality. Clone Bree with light tubers was more sensitive to ASD/CSD than clone Meulebeke with heavy tubers. Tuber vitalities averaged over burial depths and treatments were 12.8 ± 2.75% and 19.3 ± 3.22%, respectively. At Bree, the differential sensitivity among clones just failed to reach statistical significance ($p = 0.06$). Most likely, the lower efficacy levels obtained for ASD and CSD at Bree (from 10.0 up to 18.7 percentage points lower relative to Mol, averaged over burial depths) may have obscured differential sensitivity to ASD and CSD. The lower sensitivity of large tubers to soil disinfestation might be explained by the higher reserves of starch and nutrients, the higher amount of axillary buds, and the higher tuber longevity [54–57].

Hypothesis 3 was clearly supported. Indeed, the efficacy of ASD with fresh grass, Herbie®, or CSD with metam-sodium clearly differed among locations. For ASD with grass and Herbie®, and CSD with metam-sodium, tuber vitality (averaged over burial depths) was respectively 34.6, 83.1, and 75.7% lower at Mol compared to Bree. Also in the control plots, tuber vitality (averaged over burial depths) was 31.8% lower at Mol. The lower tuber vitality in the control plots at Mol might be explained by thermal injury through solarization effects. At Mol, the plots were only covered with a transparent VIF foil. However, at Bree, plots were tarped with VIF foil and a standard light-tight black-on-white coextruded silage foil that protects the VIF foil against bird damage. Given that the light transmission through the cover was not blocked at Mol and that sunshine duration (mean daily sunshine of approximately 9.5 h) and solar radiation (mean daily radiation of approximately 2100 J cm^{-2}) were similar at both locations, the soil temperature under the VIF-foil was probably higher at Mol. However, this location effect cannot solely be explained by the differential degree of solar soil warming (solarization), as the efficacy of solarization against living organisms typically decreases with increasing soil depth. El-Keblawy and Al-Hammadi [58] observed that the effectivity of soil solarization performed under high solar radiation conditions in the United Arab Emirates was less at greater depths. After solarization, seed germination of *Portulaca oleracea* L. (common purslane) seeds buried at a depth of 2.5 and 15 cm was 23.3 and 55.9%, respectively. Hence, thermal injury through solarization may only be relevant for superficially buried tubers.

Apart from the stronger solarization effect at Mol, the higher efficacies of ASD and CSD obtained in Mol can also be explained by the 49% higher gravimetric soil moisture

content in the 0–30 cm top layer at Mol compared with Bree (24.7% vs. 16.6%). According to Butler et al. [59], a high soil moisture content is needed to maintain the anaerobic conditions in the soil during ASD. Higher soil water availabilities lead to higher microbial activity and enzyme activity [60], and hence, higher production of phytotoxic chemical substances. Furthermore, moist soils tend to adsorb fewer chemical compounds than dry soils because water molecules compete with chemical compounds for the binding sites [61]. Hence, in moist soils, more phytotoxic gases are readily available to affect *C. esculentus* tuber vitality. For successful ASD, it is argued that soil moisture should be at or close to field capacity [18,19,22]. The higher soil moisture content at Mol might improve water availability for soil organisms. Looking at our gas measurements, there is also some evidence that the higher moisture content led to a less oxygenated soil at Mol. At Mol, soil oxygen concentration (at 20 cm depth, averaged over the treatments) on days 7, 13, and 29 after the experimental set-up was 0.134, 0.019, and 0.225%, respectively. At Bree, 6, 15, and 23 days after the experimental set-up, these values were 1.200, 0.294, and 0.591%, respectively. Higher soil moisture contents are also beneficial for the efficacy of metam-sodium based CSD, as metam-sodium needs to be converted into its active metabolite, methyl isothiocyanate (MITC), by chemical hydrolysis [62,63].

Soil texture might be another explanation for the differential performance of ASD between locations. Higher efficacies were obtained on the experimental site with the coarsest soil texture, namely Mol (sandy soil, 1.15% organic carbon). According to Runia et al. [30], ASD with grass is very effective on light sandy soils, but not on marine clay soils. Moreover, compared to the sandy soil in Bree (1.7% organic carbon, sandy loam), the sandy soil at Mol (1.15% organic carbon, sand) also contains a lower amount of the colloidal particles clay and humus, which largely determine the adsorptive capacity of the soil. As a result, bioactive compounds produced during ASD and CSD are less adsorbed and hence more freely available to affect *C. esculentus* tubers.

The results clearly indicate that the efficacy of ASD and CSD differs among the two locations. Therefore, repeating the experiments in another year could provide more insight into the differential performance of ASD strategies and their consistency across environments and years. Nevertheless, we observed that ASD might have the potential to be a good alternative to CSD with metam-sodium. Moreover, it would be interesting to investigate the effect of multiple dosage rates of fresh grass clippings and Herbie® on different soil types. In Western European conditions, ASD must be performed during the summer months to obtain high soil temperatures which are necessary for ASD. For example, ASD could be performed during August, after the harvest of cereals (wheat, barley, etc.). Before large scale implementation of ASD, the environmental fate of nutrients (leaching potential of nitrates in particular) added to the ASD plots should be investigated. During ASD, a lot of nutrients (especially nitrogen) are added to the soil. For example, a dose of 25 tonnes ha^{-1} Herbie® corresponds to an application of 974 kg N ha^{-1}. A dose of 80 tonnes ha^{-1} fresh grass clippings corresponded to an application of 663 kg N ha^{-1} at Bree and 424 kg N ha^{-1} at Mol. So far, the mineralization rates of nitrogen contained in Herbie® or grass are not known. To reduce leaching of nitrates available from mineralization of ASD organic carbon sources, it is highly recommended to sow a nitrogen catch crop before mid-September. Additionally, a catch crop can suppress shoots emerging from deeply positioned tubers that survived the ASD treatment. Many tubers buried deeper than the incorporation depth of the organic matter will probably survive the treatment. Generally, several percentages (up to 10% or even more) of the tubers are located below the 25 cm incorporation depth [64,65]. Additionally, ASD (even when performed in the most favorable conditions) never led to 100% control of tubers in the incorporation zone. As a result, a control of 100% is very unlikely. Thus, after ASD, regular field monitoring in subsequent years is still required.

5. Conclusions

Anaerobic soil disinfestation with fresh grass and Herbie® in particular can at least be as effective as chemical soil disinfestation with metam-sodium. Anaerobic soil disinfestation was most effective on small tubers and in light textured moist soils. The highest efficacy of ASD was obtained when the carbon source had a C:N ratio of approximately 10 and the S content was high (around 2.6 g S per kg DM), as was the case for Herbie®. The highest reductions in tuber vitality (93.8%) and performance consistency were obtained after ASD with Herbie®. However, it is important to mention that the very high control rates (>90%) were only obtained under the aforementioned conditions and that efficacies derived from bagged tubers (30 per bag) buried at three particular depths up to 30 cm may overestimate efficacies obtainable in infested fields. In naturally infested fields, tubers are randomly distributed in the topsoil and may occur at greater depths (up to a depth of 45 cm) [64,65].

To maximize the performance of ASD against the soil tuber bank of *C. esculentus*, ASD should be performed when the soil is warm and moist (soil moisture content of 20–25% or more, irrigation might be necessary) and with a carbon source with a low C:N ratio and high S content incorporated to a depth of at least 25 cm. Cloddy soil should be avoided (rototill when needed), as tubers enclosed in hard clods may be protected from the phytotoxic effects of chemicals formed during ASD as a result of reduced gas diffusion. In Northwest Europe, ASD could, for example, be performed after the harvest of winter cereal (end of July–early August) or a fast-growing vegetable crop such as spinach (*Spinacia oleracea* L.) or cauliflower (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *botrytis*). To mitigate nitrate leaching, ASD should be followed by the installation of a competitive nitrogen catch crop or winter cereal. As complete control of *C. esculentus* tubers is very unlikely, it is important to keep monitoring the infected field in the years following ASD and to install competitive row crops that allow visual inspection and selective control of emerging *C. esculentus* shoots, for example, silage maize. To foster the implementation of ASD by farmers, future research should focus on the evaluation of performance consistency across environments and years, optimization (dose rate and incorporation depth), and nitrate leaching mitigation.

Author Contributions: J.F.: Conceptualization, methodology, analysis (lead), writing—original draft, writing—reviewing and editing. D.R.: Writing—reviewing and editing. W.D.S.: analysis, methodology, resources. S.C.: Conceptualization, methodology, resources. S.P.: Conceptualization, methodology, resources. G.V.d.V.: Conceptualization, methodology, resources. B.D.C.: Conceptualization (lead), methodology, writing—reviewing and editing (lead), resources. All authors have read and agreed to the published version of the manuscript.

Funding: Both this research and the APC were funded by Flanders Innovation and Entrepreneurship (VLAIO), grant number AIO.LAN.2021.0003.01.

Institutional Review Board Statement: Not applicable.

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

Acknowledgments: This study was financially supported by Flanders Innovation and Entrepreneurship (VLAIO). Special thanks to the contractors (De Ceuster NV and Loonbedrijf Seelen B.V.) as well as the technical staff of Ghent University, Hogeschool Gent, the Educational Research Center (PVL Bocholt), and the Hooibeekhoeve (located at Geel) for the practical aid during the experiments.

Conflicts of Interest: The authors declare that there is no conflicts of interest.

References

1. Holm, L.G.; Plucknett, D.L.; Pancho, J.V.; Herberger, J.P. *The World's Worst Weeds. Distribution and Biology*; University Press of Hawaii: Honolulu, HI, USA, 1977; ISBN 978-082-480-295-0.
2. Bohren, C.; Wirth, J. Souchet comestible (*Cyperus esculentus* L.): Situation actuelle en Suisse. *Rech. Agron. Suisse* **2016**, *4*, 460–467.
3. Total, R.; Collet, L.; Heyer, J.; Keller, M. Yield losses in vegetable and arable crops caused by yellow nutsedge (*Cyperus esculentus*) in farmers' fields in Switzerland. *Julius-Kühn-Archiv* **2018**, *458*, 473–477.

4. Stoller, E.W.; Wax, L.M.; Slife, F.W. Yellow nutsedge (*Cyperus esculentus*) competition and control in corn (*Zea mays*). *Weed Sci.* **1979**, *27*, 32–37. [CrossRef]
5. Stoller, E.W. *Yellow Nut Sedge: A Menace in the Corn Belt (No. 1642)*; US Department of Agriculture, Agricultural Research Service: Washington, DC, USA, 1981.
6. Stoller, E.W.; Woolley, J.T. The effects of light and temperature on yellow nutsedge (*Cyperus esculentus*) basal-bulb formation. *Weed Sci.* **1983**, *31*, 148–152. [CrossRef]
7. De Cauwer, B.; de Ryck, S.; Claerhout, S.; Biesemans, N.; Reheul, D. Differences in growth and herbicide sensitivity among *Cyperus esculentus* clones found in Belgian maize fields. *Weed Res.* **2017**, *57*, 234–246. [CrossRef]
8. Demeyere, A. Praktijkgids Gewasbescherming. Module IPM Akkerbouw. Departement Landbouw en Visserij. Available online: https://www.vlaanderen.be/publicaties/praktijkgids-gewasbescherming-module-ipm-akkerbouw (accessed on 6 April 2023).
9. Jordan-Molero, J.; Stoller, E. Seasonal development of yellow and purple nutsedges (*Cyperus esculentus* and *C. rotundus*) in Illinois. *Weed Sci.* **1978**, *26*, 614–618. [CrossRef]
10. De Ryck, S.; Reheul, D.; de Cauwer, B. Impacts of herbicide sequences and vertical tuber distribution on the chemical control of yellow nutsedge (*Cyperus esculentus* L.). *Weed Res.* **2021**, *61*, 454–464. [CrossRef]
11. Follak, S.; Belz, R.; Bohren, C.; de Castro, O.; del Guacchio, E.; Pascual-Seva, N.; Schwarz, M.; Verloove, F.; Essl, F. Biological flora of Central Europe: *Cyperus esculentus* L. *Perspect. Plant Ecol. Evol. Syst.* **2016**, *23*, 33–51. [CrossRef]
12. Bohren, C.; Wirth, J. Implementation of control strategies against yellow nutsedge (*Cyperus esculentus* L.) into practice. *Julius-Kühn-Archiv* **2018**, *458*, 189–197.
13. Di Gioia, F.; Ozores-Hampton, M.; Hong, J.; Kokalis-Burelle, N.; Albano, J.; Zhao, X.; Black, Z.; Gao, Z.; Wilson, C.; Thomas, J.; et al. The effects of anaerobic soil disinfestation on weed and nematode control, fruit yield, and quality of Florida fresh-market tomato. *Hortscience* **2016**, *51*, 703–711. [CrossRef]
14. Riemens, M.; Huiting, H.; Heijting, S. Geïntegreerde Bestrijding van Knolcyperus: Resultaten van een Literatuurstudie Naar Alternatieven voor Monam. Available online: https://edepot.wur.nl/423464 (accessed on 11 April 2023).
15. Johnson, W.C., III; Mullinix, B.G., Jr. Yellow nutsedge (*Cyperus esculentus*) control with metham-sodium in transplanted cantaloupe (*Cucumis melo*). *Crop Prot.* **2007**, *26*, 867–871. [CrossRef]
16. Bui, H.X.; Desaeger, J.A. Volatile compounds as potential bio-fumigants against plant-parasitic nematodes—A mini review. *J. Nematol.* **2021**, *53*, 1–12. [CrossRef] [PubMed]
17. Shrestha, U.; Rosskopf, E.; Butler, D. Effect of anaerobic soil disinfestation amendment type and C: N ratio on *Cyperus esculentus* tuber sprouting, growth and reproduction. *Weed Res.* **2018**, *58*, 379–388. [CrossRef]
18. Khadka, R.B.; Cardina, J.; Miller, S.A. Perspectives on anaerobic soil disinfestation for weed management. *J. Integr. Pest Manag.* **2021**, *12*, 32. [CrossRef]
19. Blok, W.J.; Lamers, J.G.; Termorshuizen, A.J.; Bollen, G.J. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology* **2000**, *90*, 253–259. [CrossRef] [PubMed]
20. Momma, N.; Yamamoto, K.; Simandi, P.; Shishido, M. Role of organic acids in the mechanisms of biological soil disinfestation (BSD). *J. Gen. Plant Pathol.* **2006**, *72*, 247–252. [CrossRef]
21. Molendijk, L.P.G.; Bleeker, P.O.; Lamers, J.G.; Runia, W.T. Perspectives of anaerobic soil disinfestation. In Proceedings of the International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation, Leuven, Belgium, 13–18 September 2009.
22. Pakeman, R.J.; Small, J.L.; Torvell, L. Edaphic factors influence the longevity of seeds in the soil. *Plant Ecol.* **2012**, *213*, 57–65. [CrossRef]
23. Muramoto, J.; Shennan, C.; Zavatta, M.; Baird, G.; Toyama, L.; Mazzola, M. Effect of anaerobic soil disinfestation and mustard seed meal for control of charcoal rot in California strawberries. *Int. J. Fruit Sci.* **2016**, *16*, 59–70. [CrossRef]
24. Vecchia, L.; di Gioia, F.; Ferrante, A.; Hong, J.C.; White, C.; Rosskopf, E.N. Integrating cover crops as a source of carbon for anaerobic soil disinfestation. *Agronomy* **2020**, *10*, 1614. [CrossRef]
25. Strauss, S.; Kluepfel, D. Anaerobic soil disinfestation: A chemical-independent approach to pre-plant control of plant pathogens. *J. Integr. Agric.* **2015**, *14*, 2309–2318.
26. Shrestha, U.; Augé, R.M.; Butler, D.M. A meta-analysis of the impact of anaerobic soil disinfestation on pest suppression and yield of horticultural crops. *Front. Plant Sci.* **2016**, *7*, 1254. [CrossRef] [PubMed]
27. Lamers, J.; Wanten, P.; Blok, W. Biological soil disinfestation: A safe and effective approach for controlling soilborne pests and diseases. *Agroindustria* **2004**, *3*, 289–291.
28. Liu, D.; Samtani, J.B.; Johnson, C.S.; Butler, D.M.; Derr, J. Weed control assessment of various carbon sources for anaerobic soil disinfestation. *Int. J. Fruit Sci.* **2020**, *20*, 1005–1018. [CrossRef]
29. Statbel. Bodemgebruik. Available online: https://statbel.fgov.be/nl/themas/leefmilieu/grond/bodemgebruik#figures (accessed on 17 March 2023).
30. Runia, W.T.; Molendijk, L.P.; Ludeking, D.J.; Schomaker, C.H. Improvement of anaerobic soil disinfestation. *Comm. Agric. Appl. Biol. Sci.* **2012**, *77*, 753–762.
31. Meints, H.; Feil, H. Method of Biological Soil Decontamination. US 8852503 B2, 7 October 2014.
32. Ludeking, D.; Termorshuizen, A.; Wubben, J.; van der Wurff, A.; Streminska, M.; van der Helm, F. *Biologische Grondontsmetting Met Herbie ('Bodemresetten') als Alternatief voor Stomen*; Wageningen UR Glastuinbouw: Wageningen, The Netherlands, 2013.

33. Starbuck, C.J. *Grass Clippings, Compost and Mulch: Questions and Answers*; Mu Guide; University of Missouri-Columbia: Columbia, MO, USA, 2022.
34. Hoek, J.; Visser, J.H.M.; Molendijk, L.P.G. Bestrijding van Knolcyperus Knolletjes door Anaerobe Grondontsmetting Met Gras en Met Herbie ("Bodem-Resetten"). Available online: https://edepot.wur.nl/573981 (accessed on 11 April 2023).
35. Minnesota Stormwater Manual. Soil Water Storage Properties. Available online: https://stormwater.pca.state.mn.us/index.php/Soil_water_storage_properties (accessed on 24 July 2023).
36. Saxton, K.E.; Rawls, W.J. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1569–1578. [CrossRef]
37. DOV. Soil Map of Belgium. Available online: https://www.dov.vlaanderen.be (accessed on 27 July 2023).
38. Compendium Voor Monsterneming en Analyse in Uitvoering van Het Materialendecreet en Het Bodemdecreet. Elementen. Available online: https://reflabos.vito.be/2023/CMA_2_IV_19.pdf (accessed on 27 July 2023).
39. *ISO 10694:1995*; Soil Quality—Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis). ISO: Geneva, Switzerland, 1995. Available online: https://www.iso.org/standard/18782.html (accessed on 27 July 2023).
40. *ISO 16634-1:2008*; Food Products—Determination of the Total Nitrogen Content by Combustion According to the Dumas Principle and Calculation of the Crude Protein Content—Part 1: Oilseeds and Animal Feeding Stuffs. ISO: Geneva, Switzerland, 2008. Available online: https://www.iso.org/standard/46328.html (accessed on 27 July 2023).
41. De Ryck, S.; Reheul, D.; de Riek, J.; de Keyser, E.; de Cauwer, B. Genetic and morphological variation of Belgian *Cyperus esculentus* L. clonal populations and their significance for integrated management. *Agronomy* **2023**, *13*, 572.
42. Eijkelkamp. Bodemzuurstofmeter Set. Available online: https://www.royaleijkelkamp.com/nl/producten/veldmeetapparatuur/zuurstof-in-de-bodem/analyse/bodemzuurstofmeter-set/ (accessed on 26 April 2023).
43. Priyashantha, A.H.; Attanayake, R.N. Can anaerobic soil disinfestation (ASD) be a game changer in tropical agriculture? *Pathogens* **2021**, *10*, 133. [CrossRef] [PubMed]
44. Ruf, M.; Brunner, I. Vitality of tree fine roots: Reevaluation of the tetrazolium test. *Tree Physiol.* **2003**, *23*, 257–263. [CrossRef]
45. R Core Team. R: A Language and Environment for Statistical Computing. Available online: https://www.R-project.org/ (accessed on 3 May 2023).
46. Gao, S.; Tanji, K.K.; Scardaci, S.C. Impact of rice straw incorporation on soil redox status and sulfide toxicity. *Agron. J.* **2004**, *96*, 70–76. [CrossRef]
47. Achmon, Y.; Fernández-Bayo, J.D.; Hernandez, K.; McCurry, D.G.; Harrold, D.R.; Su, J.; Simmons, C.W. Weed seed inactivation in soil mesocosms via biosolarization with mature compost and tomato processing waste amendments. *Pest Manag. Sci.* **2017**, *73*, 862–873. [CrossRef]
48. Yang, Z.; Wang, X.; Feng, J.; Zhu, S. Biological functions of hydrogen sulfide in plants. *Int. J. Mol. Sci.* **2022**, *23*, 15107. [CrossRef] [PubMed]
49. Li, Y.; Park, S.Y.; Zhu, J. Solid-state anaerobic digestion for methane production from organic waste. *Renew. Sustain. Energy Rev.* **2011**, *15*, 821–826. [CrossRef]
50. Pan, W.L.; Madsen, I.J.; Bolton, R.P.; Graves, L.; Sistrunk, T. Ammonia/ammonium toxicity root symptoms induced by inorganic and organic fertilizers and placement. *J. Agron.* **2016**, *108*, 2485–2492. [CrossRef]
51. Singh, G.; Ward, B.K.; Wechter, W.P.; Katawczik, M.L.; Farmaha, B.S.; Suseela, V.; Cutulle, M.A. Assessment of agro-industrial wastes as a carbon source in anaerobic disinfestation of soil contaminated with weed seeds and phytopathogenic bacterium (*Ralstonia solanacearum*) in tomato (*Solanum lycopersicum*). *J. Agric. Sci. Technol.* **2022**, *2*, 769–779.
52. Visser, J.H.M.; Molendijk, L.P.G.; Feil, H.; Meints, H.; van Beers, T. *Bodem Resetten: Innovatieve Anaerobe Grondontsmetting (ASD) Tegen Schadelijke Bodemorganismen*; Wageningen University & Research: Wageningen, The Netherlands, 2017.
53. De Brabander, D.; de Campenaere, S.; Ryckaert, I. Vlaamse Overheid, Departement Landbouw en Visserij. Available online: https://publicaties.vlaanderen.be/view-file/38135 (accessed on 27 June 2023).
54. Santos, B.M.; Morales-Payan, J.P.; Stall, W.M.; Bewick, T.A.; Shilling, D.G. Effects of shading on the growth of nutsedges (*Cyperus* spp.). *Weed Sci.* **1997**, *45*, 670–673.
55. Stoller, E.W.; Nema, D.P.; Bhan, V.M. Yellow nutsedge tuber germination and seedling development. *Weed Sci.* **1972**, *20*, 93–97. [CrossRef]
56. Stoller, E.W.; Wax, L.M. Yellow nutsedge shoot emergence and tuber longevity. *Weed Sci.* **1973**, *21*, 76–81. [CrossRef]
57. Thullen, R.J.; Keeley, P.E. Seed production and germination in *Cyperus esculentus* and *C. rotundus*. *Weed Sci.* **1979**, *27*, 502–505. [CrossRef]
58. El-Keblawy, A.; Al-Hammadi, F. Soil amendments enhance soil solarisation efficiency in controlling weeds under the environment of the United Arab Emerites. In Proceedings of the 16th Australian Weeds Conference, Cairns Convention Centre, Cairns, QLD, Australia, 18–22 May 2008.
59. Butler, D.M.; Kokalis-Burelle, N.; Muramoto, J.; Shennan, C.; McCollum, T.G.; Rosskopf, E.N. Impact of anaerobic soil disinfestation combined with soil solarization on plant–parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. *Crop Prot.* **2012**, *39*, 33–40. [CrossRef]
60. Stark, J.M.; Firestone, M.K. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Appl. Environ. Microb.* **1995**, *61*, 218–221.
61. Calvet, R. Adsorption of organic chemicals in soils. *Environ. Health Perspect.* **1989**, *83*, 145–177. [CrossRef] [PubMed]
62. Turner, N.J.; Corden, M.E. Decomposition of sodium N-methyldithiocarbamate in soil. *Phytopathology* **1963**, *53*, 1388–1394.

63. El Hadiri, N.; Ammati, M.; Chgoura, M.; Mounir, K. Behavior of methyl isothiocyanate in soils under field conditions in Morocco. *Chemosphere* **2003**, *52*, 927–932. [CrossRef] [PubMed]
64. Tumbleson, M.E.; Kommedahl, T. Reproductive potential of *Cyperus esculentus* by tubers. *Weeds* **1961**, *9*, 646–653.
65. Stoller, E.W.; Sweet, R.D. Biology and life cycle of purple and yellow nutsedges (*Cyperus rotundus* and *C. esculentus*). *Weed Technol.* **1987**, *1*, 66–73. [CrossRef]