

Article

Arable Weeds at the Edges of Kettle Holes as Overwintering Habitat for Phytopathogenic Fungi

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Abstract: Weeds in agricultural landscapes can serve as alternate hosts for phytopathogenic fungi and promote the spatial and long-term distribution of these fungi. Especially, semi-natural habitats such as kettle holes are considered as a source of fungal pathogens because they are a permanent habitat for various weed species in arable lands. In our study, we investigated the suitability of nine different weed species and families at the edges of 18 kettle holes in two consecutive autumn/winter seasons as alternate hosts for *Fusarium* and *Alternaria*. We detected a fungal infestation with both genera on every weed species investigated with significantly higher abundances of these fungi in the second, notably wetter season. Eight weed species were described as non-host plants for *Fusarium* and *Alternaria* in agricultural landscapes in Brandenburg, Germany for the first time. In both autumn/winter periods, weeds harbored more *Alternaria* than *Fusarium*. The study revealed a high *Fusarium* species diversity in weeds and a community structure of up to 12 *Fusarium* species at the edges of kettle holes. Grasses showed the highest diversity and often the highest fungal abundances compared to herbaceous plants. Therefore, these habitats in arable lands can act as ecosystem disservice and promote the spread of fungal diseases in the surrounding crop fields.

Keywords: arable weeds; kettle holes; phytopathogenic fungi; *Fusarium*; *Alternaria*; alternative host; alternate host; non-crop host; source of infection



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1. Introduction

Filamentous fungi are mainly known to cause partially destructive diseases.

Cereals, and especially wheat (*Triticum aestivum* L.), which is one of the most economically important crops, are highly susceptible to fungal infections. In wheat, the various diseases caused by filamentous fungi lead to yield losses both prior to the harvest and afterward [1,2]. Fungal-associated diseases can be responsible for 15–20% yield losses worldwide each year [2,3].

Some of the most economically important filamentous fungi belonging to the genera *Fusarium* and *Alternaria*, which inhabit hosts in every climatic zone worldwide [4–6] and are a trigger for a wide range of plant diseases and the contamination of a great variety of crops with poisonous mycotoxins [1,7,8]. An infection with *Fusarium* spp. can be responsible for diseases such as wilts, rots, and blights [7], while the *Fusarium* head blight (FHB), caused by many different *Fusarium* species [8] is the most well-known one [9–11]. Various species of the *Alternaria* genera are mainly associated with black mold, black and grey rot, and black spot disease [1].

Concerning fungal diseases, plant debris from previous crops, particularly corn stubble, are the most important primary inocula for fungal infection in the following field season [10,12]. Filamentous fungi use them to overwinter and for sporulation [13], especially when they are the only suitable hosts left. Originating from these residues, the spores

can migrate onto the lower parts of diverse host plants such as winter and summer wheat in autumn or spring. The transportation to higher areas of the plant can happen either through wind dispersal or through raindrops, which splash the spores onto the leaves or stems [14–16].

Therefore, most studies investigate the influence that crop residues have as an inoculum for *Fusarium* spp. and *Alternaria* spp. Soil, especially humus soil with many organic compounds, is also a well-studied source of inoculation for these fungi [10,12,17–19].

Additionally, weeds growing in or next to agricultural fields play a major role in the inoculation of crops with pathogenic filamentous fungi, but they have not yet been investigated in equal depth. What is known thus far is that some arable weeds, especially plants of the family *Poaceae*, are often colonized by *Fusarium* species. Most of them were detected as asymptomatic carriers of *Fusarium* diseases because they rarely show any visible symptoms of infection. Thus, weeds have the potential to act as reservoirs for fungi, thereby increasing the hazard of an infection of the crops growing simultaneously in the field [10,19–22]. However, the importance of weeds in the *Fusarium* head blight disease cycle is not fully understood [21]. In comparison to our knowledge on the interaction of *Fusarium* fungi with non-host plants, there is a lack of information about the infection of arable weeds with species of the genera *Alternaria*.

Weeds in agricultural landscapes not only sprout alongside cultivated crops, but they also grow permanently in (semi)-natural landscape elements (NLE). NLE such as forests, hedgerows, and kettle holes often fragment agricultural landscapes into different landscape patches and cause borders, edges, and transition zones between these structures and the adjacent field [23,24]. Ries et al. [23] described the influence of NLE on the immediate and wider surroundings through different factors, such as ecological flows and the access to spatially separated resources. The resulting spatial heterogeneities play a unique role in arable lands, especially in large fields with limited crop rotation. Here, NLE act as keystone structures [25]. They offer several ecosystem services such as providing habitat, access to water or enhanced moisture, food, and shelter for numerous species of macroorganisms (e.g., ground beetles, breeding birds, amphibians, bees) and micro-organisms (e.g., fungi, bacteria) [26–31]. Water-filled kettle holes, which are natural ponds with <1 ha area [32], act as specific NLE that influence organisms in the bordering zones affected by soil moisture and air humidity. Due to the combined effect of the different types of plants in and around these structures and the increased surrounding humidity, kettle holes are regarded as hot spots for biodiversity [31,33–35] and as particularly attractive living habitats for amphibians, insects, and breeding birds. Furthermore, kettle holes in Germany are protected areas by law (Gesetz über Naturschutz und Landschaftspflege (§32 BbgNatSchG)) [36]. For this reason, harvesting of the plants growing at the edges of kettle holes and the use of herbicides next to them is prohibited. Thus, these NLE form a suitable habitat for annual and perennial grasses and herbaceous plants to survive in agricultural landscapes.

Although the kettle holes provide many ecosystem services, they are also thought to enhance fungal development and infection of agricultural fields. The diversity of plants at the edges of kettle holes offers different living conditions and microhabitats, such that a wide variety of fungi can find their special structural niche. For example, Suproniene et al. [21,22] detected that many arable weeds are alternative and asymptomatic hosts for fungi of the genera *Fusarium*. Non-crop hosts such as *Poa annua* L., *Viola arvensis* L., and *Galium aparine* L. were frequently and strongly infected by different *Fusarium* species that rarely caused any visible symptoms. Nevertheless, these plants can act as an overwintering habitat and an inoculum for more vulnerable, symptomatic plants such as wheat in the surrounding areas.

Therefore, especially, kettle holes in arable fields can be considered a source of inoculation for long-lasting infection of the neighboring fields and as an increased risk of infection and mycotoxin accumulation of crops.

The current survey aims to determine (1) if weeds at the edges of kettle holes are (heavily) infected with *Fusarium* and *Alternaria* fungi in autumn and winter, (2) which

plant species are particularly attractive hosts for *Fusarium* and *Alternaria* species, and (3) how weather conditions in these months influence the abundance and species diversity of these fungi. Based on an ArcGIS approach, we determine the relative frequency of occurrence of these non-host plants around the kettle holes investigated and conclude from this the potential for dispersal of phytopathogenic fungi from these weed plants into the surrounding crop field. For these aims, various herbaceous plants (e.g., *Urtica dioica* L., *Cirsium arvense* L., *Galium aparine* L.) and true grasses (*Poaceae*) were analyzed on their ability to provide pathogenic filamentous fungi of the genera *Fusarium* spp. and *Alternaria* spp., an alternative opportunity to overwinter on farmland and thus a permanent habitat for development, spore production, and spore release. In the autumn and winter of 2018 and 2019/2020, we examined 18 different kettle holes in total, from which we analyzed three plant samples from each.

Against this background, we assume weeds growing permanently at the edges of kettle holes in agricultural fields act as reservoirs for the infection of crops with fungal pathogens. Therefore, we discuss the balance between the provision of ecosystem services and disservices by these semi-natural landscape structures.

2. Materials and Methods

2.1. Study Site

The study site was located on farms within the Quillow catchment in Uckermark in the northeast part of the state of Brandenburg, Germany. Scientific investigations took place in the long-term research platform “AgroScapeLab Quillow” (Agricultural Landscape Laboratory Quillow, E 13°48′12″, N 53°21′59″) of the Leibniz Centre for Agricultural Landscape Research (ZALF) [37,38]. Mean values for the 1992–2013 periods were 8.6 °C for air temperature, and 563.8 mm of precipitation per year (ZALF field station, Dedelow, Germany).

Nietzsche et al. [37,38] described the topography as characterized by a hummocky landscape that was massively reshaped during the Pleistocene. The landscape and soil structure is typical for an area formed by glaciations of the Pleistocene in central continental Europe. In the area, small water bodies (<1 ha [32]) are frequent semi-natural landscape structures: more than 1500 kettle holes are located in the “AgroScapeLab Quillow”. The area is dominated by agricultural land use [32]. Therefore, land use types are composed of 74.4% agrarian fields, 10.4% grasslands, and 5.9% forest. Only 1.4% of the area is covered by up to 40 kettle holes per km², which occur in all of the land-use types mentioned before [37]. The study was implemented in winter wheat fields where maize was the preceding crop. The examined fields belonged to commercial farms; thus, the crops were managed according to standard agricultural procedures and good professional practices.

2.2. Sampling Design

The field sampling of herbaceous plants and *Poaceae* growing permanently at the edges of kettle holes took place in two consecutive years at 18 different kettle holes, 9 each year. The location of these semi-natural landscape structures within the Uckermark region, Germany is displayed in Figure 1. In 2018, we sampled in September, October, and November, while in 2019/2020, samples were taken in October, November, and January. One sampling point at each kettle hole was set, and samples of three different (the most frequent) non-crop plants were taken ($n = 27$ per month) in one square meter around this point. Both young and fresh as well as senescent and necrotic plant parts were included. The sample collection comprised a total of 162 plant samples representing 9 different plant species or families (Table 1).

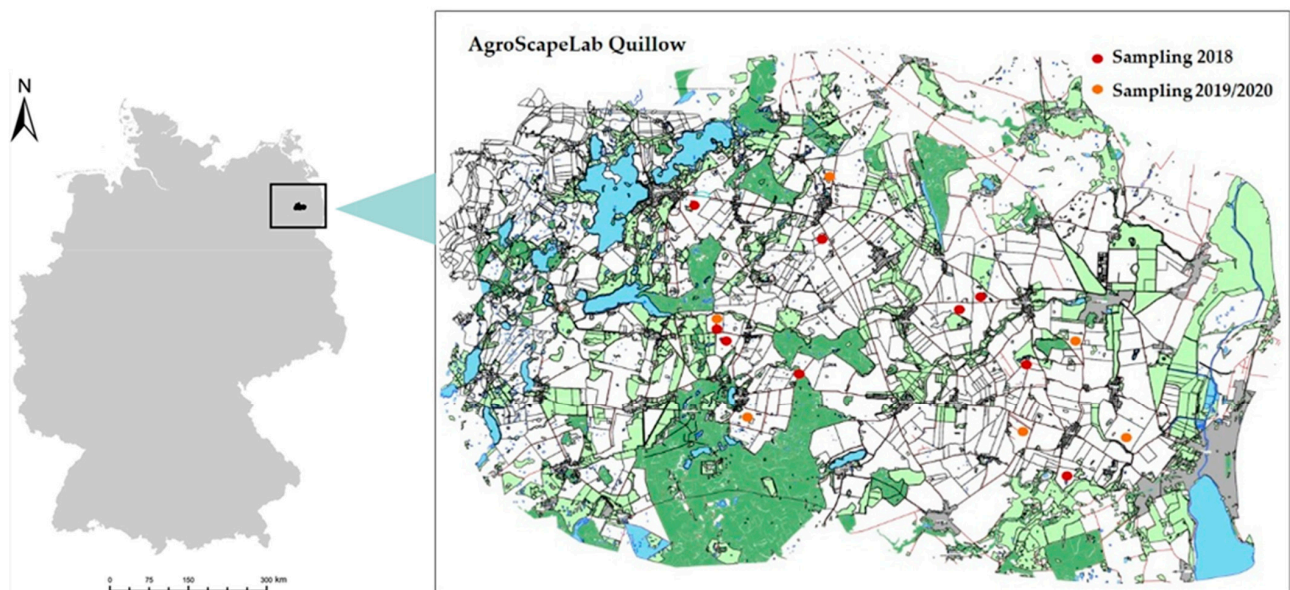


Figure 1. The location of the study region (AgroScapeLab Quillow) within the German national borders (left). Distribution fields with the kettle holes thereon, investigated in 2018 (red dots) and 2019/2020 (orange dots) within the AgroScapeLab Quillow region (right). An experimental infrastructure platform of the Leibniz Centre of Agricultural Landscape Research (ZALF) is located in Dedelow, Uckermark, State of Brandenburg, Germany.

Table 1. Overview of the different sampled weed plant species growing at the edges of kettle holes in 2018 and 2019/2020 and the number of samples of each specific weed summarized for each year.

Season	Weed Species	Number of Samples
2018	<i>Urtica dioica</i>	27
	<i>Phragmites australis</i>	12
	<i>Cirsium arvense</i>	9
	<i>Galium aparine</i>	3
	<i>Sisymbrium loeselii</i>	3
	<i>Arctium</i> sp.	3
	Grasses ¹	24
2019/2020	<i>Urtica dioica</i>	27
	<i>Cirsium arvense</i>	9
	<i>Galium aparine</i>	9
	<i>Phragmites australis</i>	3
	<i>Rumex</i> sp.	3
	<i>Tanacetum vulgare</i>	3
	Grasses ¹	27

¹ The grass samples consisted of different proportions of *Arrhenatherum elatius*, *Bromus sterilis*, *Calamagrostis epigejos*, *Dactylis glomerata*, *Elymus repens*, *Lolium perenne*, and *Poa trivialis*.

The samples were collected in crispac bags and transported to the laboratory in cool boxes. Plant samples were stored at 4–6 °C until further investigations on the next day. Collected plants were analyzed by culture-dependent and culture-independent methods for the presence of fungi, mainly filamentous fungi of the genera *Fusarium* and *Alternaria*.

2.3. Laboratory Analyses

2.3.1. Culture-Dependent Method

For determination of the colony-forming units (cfu) per gram of fresh matter for the total fungal abundance (TOTAL_cfu/gFM), for the *Fusarium* (FUS_cfu/gFM) and the *Alternaria* abundance (ALT_cfu/gFM), potato dextrose agar (PDA; Merck, Heidelberg, Germany) supplemented with chloramphenicol and synthetic nutrient agar (SNA) [39]

were used as described in detail by Leslie and Summerell [6]. Ten pieces (about 1 cm length) of each plant sample were plated onto two PDA-containing petri dishes (diameter 9 cm) with five pieces plated on each plate. Randomly selected pieces of leaves, stems, and/or flowers of several plants per sample were arranged on the petri dishes. Before plating, the 10 plant pieces were weighed (Kern 572-35; Kern&Sohn GmbH Balingen-Frommern, Germany) to be able to calculate the colony-forming units to 1 g of plant fresh matter. Plated samples were incubated for 2 days at 24 °C in darkness and further 2 days under UV light (12 h UV light/12 h daylight) at room temperature to support the sporulation and coloration of the fungal mycelium. After 2 days, the TOTAL_cfu/gFM was calculated by counting all fungal colonies on each petri dish. The colonies of *Fusarium* (FUS_cfu/gFM) and *Alternaria* fungi (ALT_cfu/gFM) were counted 2 days after UV light treatment.

For morphological identification of the *Fusarium* species, the colonies were isolated onto a new PDA petri dish and sub-cultured onto SNA media. The cultures grown on PDA were used to analyze the species by morphological aspects such as growth rate and color of the mycelium. SNA supports each *Fusarium* fungus in developing its species-specific macro- and microspore characteristics. Plates were treated as described above with the possible exception of a longer UV light treatment of up to 10 days depending on the growth rate of the mycelium. The isolated *Fusarium* were identified to species level using light microscopy (Jenaval, Carl Zeiss, Jena, Germany). The identification was mainly based on macro- and micro-morphological characteristics already described by Leslie and Summerell [6].

2.3.2. Culture-Independent Method (qPCR Approach)

The remaining plant samples were dried at 60 °C for at least 48 h. For further analyses by qPCR, the dried samples were grounded using a vibrating cup mill RS200 (Retsch, Haan, Germany) at 1300 rpm for 1.5 min. Once milled, the material was carefully mixed, and the DNA was extracted from 250 mg according to a customized standard protocol of the NucleoSpin® Soil Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) according to the manufacturer's instruction for handling. The lyses was adapted to the plant material, and samples were centrifuged by 13,000 rpm instead of 11,000 rpm. The quantification of DNA gene copy numbers of *Fusarium* and *Alternaria* by a qPCR approach with genus-specific primers is described in detail by Müller et al. [40]. For the qPCR assay, the QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the software "QuantStudio™ 12K Flex Software v1.x" (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used. The quantification of the *Fusarium* and *Alternaria* gene copy numbers/gDM was performed using the HOT FIREPol® Probe GC qPCR Mix (Solis Biodyne, Tartu, Estonia). The PCR cycles for *Fusarium* included 95 °C for 10 min (hold stage) and 45 cycles of 95 °C for 15 s and 67 °C for 1.5 s (PCR stage). To produce standard curves, the genomic DNA of *F. graminearum* strain name "Fg486", from the microbial collection of ZALF, was used. To quantify the *Alternaria* gene copy numbers/gDM we used the TaqMan universal PCR mastermix (Applied Biosystems). Reactions were carried out under the following thermal conditions: 95 °C for 10 min (hold stage) and 45 cycles of 95 °C for 15 s, 64 °C for one 45 s (PCR stage), and 10 °C for 2 m (hold stage). The standard curves were generated by using DNA of *A. tenuissima* strain name "H50". The fungal strains used for the preparation of the standard curves were stored in a culture collection of fungi of the working group "Fungal Interactions" at the Leibniz Centre of Agricultural Landscape Research Müncheberg. All qPCR assays contained negative controls, and all measurements were performed in duplicate. The genome copy numbers were expressed in FUS_gcn/g DM for *Fusarium* fungi and as ALT_gcn/g DM for *Alternaria* fungi. See supplementary materials for further information about the primers used in the qPCR assay (Table S1).

2.4. Mapping of the Kettle Hole Edge Vegetation

The edge vegetation of six kettle holes was mapped to determine the proportion of different arable weeds growing permanently at the edges. The selected kettle holes cover

different size ranges (from 515 to 4493 m²) and were characterized by different dominant vegetation types covering different succession states, such as “edge type with reed canary grass”, “full reed type with sedges”, or “wood type”. The manual mapping was based on orthomosaics created with images collected by a UAS (Unmanned Aerial System) directly before harvest in June 2020 (Figure 2). For the UAS flight campaign, we used a Phantom 4 RTK (DJI) carrying a 20 Megapixel CMOS sensor with a fixed 8.8 mm lens. The flight missions were planned using the DJI GS RTK app integrated mission planning software. All six kettle holes were flown over with parallel flight tracks between 25 and 35 m altitude and nadir camera orientation. A high relative position accuracy of the images was achieved by connecting to the mobile station D-RTK 2 and additional ground control points (GCPs) around the kettle holes. All captured UAS images were quality controlled and processed in a photogrammetric SfM workflow using Agisoft Metashape Professional 1.6.2 build 6205 (Agisoft LLC) to compute an orthomosaic.



Figure 2. Cutout of the vegetation growing at the edge of one kettle hole investigated in 2019/2020. Image collected by an Unmanned Aerial System (UAS) in June 2020. Orthomosaics were created with this image by using Agisoft Metashape Professional 1.6.2 build 6205.

Based on the orthomosaics, we mapped the edge vegetation of the kettle holes manually in QGIS (QGIS.org, 2021. QGIS Geographic Information System. QGIS Association. <http://www.qgis.org>, accessed on 15 December 2021). The marginal vegetation was divided into five categories, namely herbs, grasses, herbs and grasses, woody plants, and grasses and woody plants. The five vegetation categories were visually distinguished from the surrounding patches by color, texture, and shape and based on our experience in previous studies [41]. The assignment to a vegetation category was based on its dominance. If it was a mixed stand, the vegetation patch was assigned to a mixed category, such as herbs and grasses. Vegetation categories were only mapped in the edge area of the kettle hole, which we defined by a 3 m buffer from the boundary of the kettle hole into the kettle hole. The boundary of the kettle hole was defined as a sharp edge between the natural vegetation of the kettle hole and the adjacent cultivated fields.

2.5. Statistics

The abundance data (cfu/gFM and gcn/gDM) were tested for normal distribution via the Kolmogorov–Smirnov test. The abundance of total fungi, *Fusarium* and *Alternaria*, between the 2018 and 2019/2020 seasons was compared by t test. The differences in fungal quantities between the herbaceous plants and the plants of the family of *Poaceae* (gcn/gDM) were analyzed by the Wilcoxon–Mann–Whitney test, while the different weed species (gcn/gDM) were compared by Kruskal–Wallis test. A logarithmic transformation LOG (x + 1) was applied to the data of the culture-independent qPCR approach for the visualization of the gcn/gDM of fungal abundances as boxplots. The midline represents

the median; the upper and lower limits of the boxes the third and first quartile. In all figures, asterisk brackets indicate significant differences between parameters investigated (p values < 0.05). All statistical tests were realized with SPSS (IBM SPSS Statistics V 22.0).

3. Results

3.1. Abundance of Total Fungi, *Fusarium*, and *Alternaria* in the Autumn/Winter Months Influenced by Weather Conditions

For the following analyses, the data of the 18 kettle holes, investigated over 2 years, were pooled together, and the collected data of all month and years were combined. The abundance of *Fusarium* and *Alternaria* fungi in comparison to the total fungal abundance was analyzed by the culture-dependent method through determining the cfu/gFM.

Our results clearly showed that weed plants at the edges of the kettle holes harbor phytopathogenic fungi in both autumn and winter seasons. However, we also detected significant differences between the investigated years for TOTAL_cfu/gFM for FUS_cfu/gFM and for ALT_cfu/gFM ($* p < 0.05$) (Figure 3). In the second season investigated, the mean *Fusarium* abundance was six-fold higher than in the first season. The mean abundance of *Alternaria* fungi was 23 times higher in 2019/2020, compared to 2018 (Figure 3). In 2018, *Fusarium* and *Alternaria* each accounted for 11.3% of the total fungal load. In 2019/2020, *Alternaria* accounted for 26.9% and *Fusarium* for 44.7%.

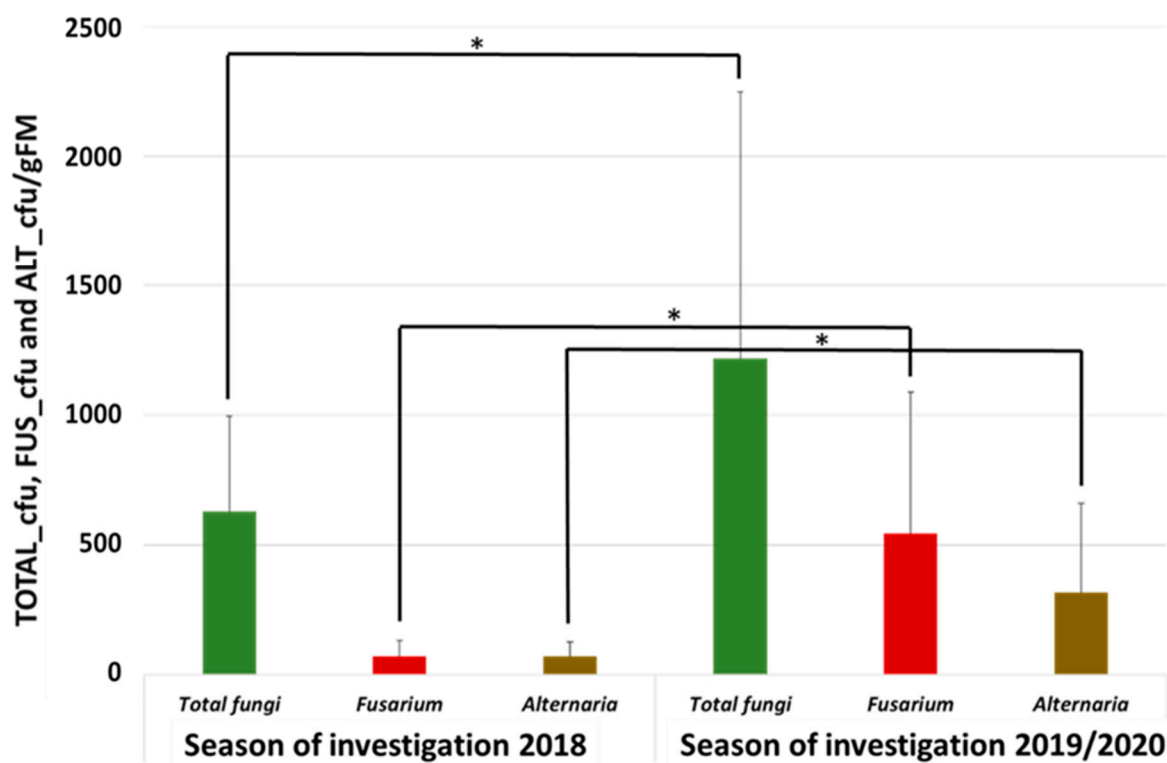


Figure 3. Mean values of the colony-forming units of total fungi (TOTAL_cfu/gFM), *Fusarium* (FUS_cfu/gFM) and *Alternaria* (ALT_cfu/gFM) in 2018 ($n = 81$) and 2019/2020 ($n = 81$) isolated from overwintered plant material at the edges of nine kettle holes in the AgroScapeLab Quillow. The plant species were summarized in Table 1, and the fungal abundance (expressed as cfu/gFM) was averaged over all plant samples in each year. The mean values are plotted with their standard deviation of the mean. Asterisk brackets indicate significant differences ($* p < 0.05$).

The mean annual temperatures in 2018 and 2019 were nearly identical: 10.4 and 10.7 °C, respectively. Compared to the long-time average of 8.6 °C for air temperature (mean values for 1992–2013), both years were about 2 °C warmer (Table 2). In contrast, the annual precipitation in 2018 was 24.7% lower than in 2019 (346.1 mm in 2018; 459.8 mm in 2019). However, both years were drier than the long-term average: 38.6% less precipitation

in 2018 and 18.4% less in 2019 (Table 2). A possible explanation for the different levels of fungal contamination of all weed plants could be the different microclimatic conditions in the autumn and winter months of the two years. The significantly higher amount of precipitation in these months in 2019/2020 forced the high abundance of *Fusarium* and *Alternaria* fungi.

Table 2. Monthly average air temperature (°C), monthly sum of precipitation (mm), annual mean values as well as the long-time average from 1992 until 2013 (Long-time av.) during and before/after the sampling months in 2018, 2019, and 2020. All data were measured by the ZALF weather station at Dedelow (see Figure 1). The values of the sampling months and the month before the sampling of this study are highlighted.

Month	Air Temperature (Mean in °C)			Month	Precipitation (Sum in mm)		
	2018	2019	2020		2018	2019	2020
January	2.7	1.1	4.4	January	70.4	37.5	23.4
February	−2.2	4.2	5.6	February	13.6	15.2	40.8
March	0.7	6.6	4.7	March	39.5	20.4	24.5
April	11.9	9.6	9.1	April	30.5	6.7	17.5
May	16.2	11.7	12.1	May	14	47.8	18.3
June	18.4	20.9	18.1	June	15.9	86.6	44.3
July	20.8	18.9	17.7	July	50.3	51.7	38.4
August	20.7	20.1	20.2	August	21.3	18.7	65.4
September	15.7	14.6	14.7	September	10	61.4	59.6
October	10.4	10.6	10.9	October	15.5	47.1	49
November	4.6	5.9	n.a.	November	17.9	50.3	n.a.
December	4.0	3.9	n.a.	December	47.2	16.4	n.a.
Annual mean	10.4	10.7	n.a.	Sum year	346.1	459.8	n.a.
Long-time average		8.6		Long-time average		563.8	

3.2. Abundance of *Fusarium* and *Alternaria* on *Poaceae* and *Herbaceous* Plants (qPCR Approach)

For these investigations, the plant samples were divided into two groups, plants of the family of *Poaceae* (different grass species and reed) and the group of herbaceous plants, and were analyzed separately. In 2018, 44% of the sampled plants belonged to the family of *Poaceae* ($n = 36$), and 56% belonged to herbaceous plants ($n = 45$). In 2019/2020, 37% belonged to the family of *Poaceae* ($n = 30$), and 63% belonged to herbaceous plants ($n = 51$). The number of herbaceous plants sampled and examined predominated in both seasons.

Weeds of the family of *Poaceae* as well as herbaceous plants were more colonized by *Alternaria* (ALT_gcn/gDM) fungi than by *Fusarium* (FUS_gcn/gDM) in both years of investigation (Figure 4). We also observed a stronger infection with both phytopathogenic fungi in the wetter season in 2019/2020, independently of the type of plants examined. In 2018, there were no significant differences between plants of the family *Poaceae* and herbaceous plants in the *Fusarium* abundance. Regarding *Alternaria*, herbaceous plants were significantly higher colonized in 2018. In 2019/2020, we detected a significantly higher *Fusarium* colonization on plants of the family *Poaceae* compared to the herbaceous plants: they were 15 times higher infected with *Fusarium* fungi (Figure 4). For *Alternaria*, no significant difference was found.

For further analyses, the abundance of *Fusarium* and *Alternaria* fungi was studied on different single plant species of herbaceous plants and plants of the family *Poaceae*. Herbaceous plants were investigated by considering three plant species that occurred in both years: *Urtica dioica*, *Cirsium arvense* and *Galium aparine*.

With regard to the abundance of *Fusarium* gcn/gDM, in the 2019/2020 winter season, significantly higher fungal colonization than in 2018 was detected on all three weed species investigated. The highest infestation was detected on *Galium aparine* (Figure 5). These differ significantly from the lower *Fusarium* abundances of *Urtica dioica* and *Cirsium arvense*. In 2018, again *Galium aparine* showed the highest fungal abundance. At the same time, the highest *Alternaria* abundance was found on *Galium aparine* in both years. Thus, this weed

species proves to be a frequent host plant for both phytopathogenic fungal genera in both years. Concerning *Alternaria* abundances, differences between the arable weeds in both seasons are to be noted—*Cirsium arvense* and *Urtica dioica* were less colonized by *Alternaria* fungi (Figure 5). Overall, all three weed species were found to be highly contaminated host plants for fungi. *Alternaria* fungi were detected in higher abundances on the three weeds; at the same time, *Galium aparine* was the most severely infected weed species. However, the infection of all three weed plants was higher in the wetter autumn/winter season of 2019/2020 than in the autumn/winter season of 2018.

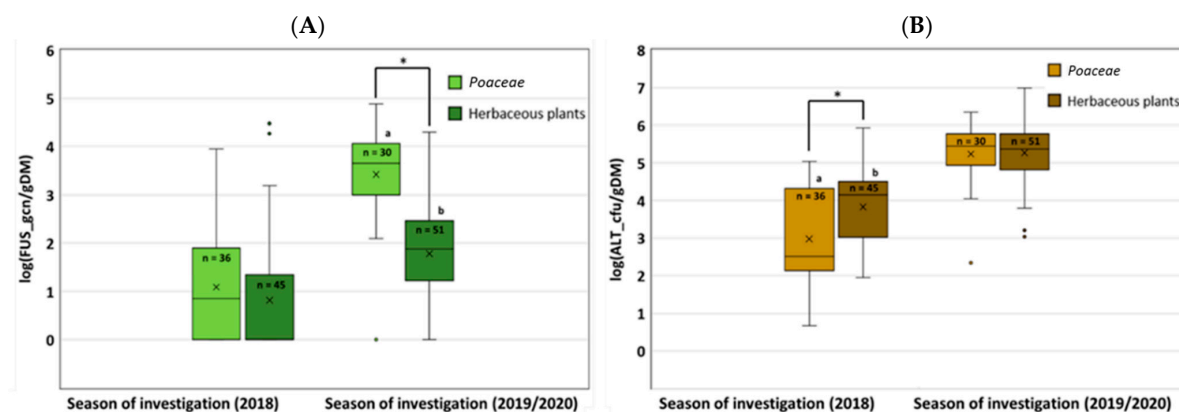


Figure 4. Abundances of *Fusarium* (A) and *Alternaria* (B) fungi expressed as LOG (x + 1) (gene copy numbers) of *Fusarium* (FUS_gcn/gDM) and *Alternaria* (ALT_gcn/gDM) on weed plants of the *Poaceae* family and of herbaceous plants in 2018 and 2019/2020. The different weed plants were sampled at edges of all kettle holes. The midline of the boxplots represents the median; x is the mean value; the upper and lower limits of the boxes are the third and first quartiles. Asterisk brackets indicate significant differences between parameters investigated (* *p*-values < 0.05; ** is an outlier). a and b highlight that there is a significant difference (*p* < 0.05) between the samples to be found.

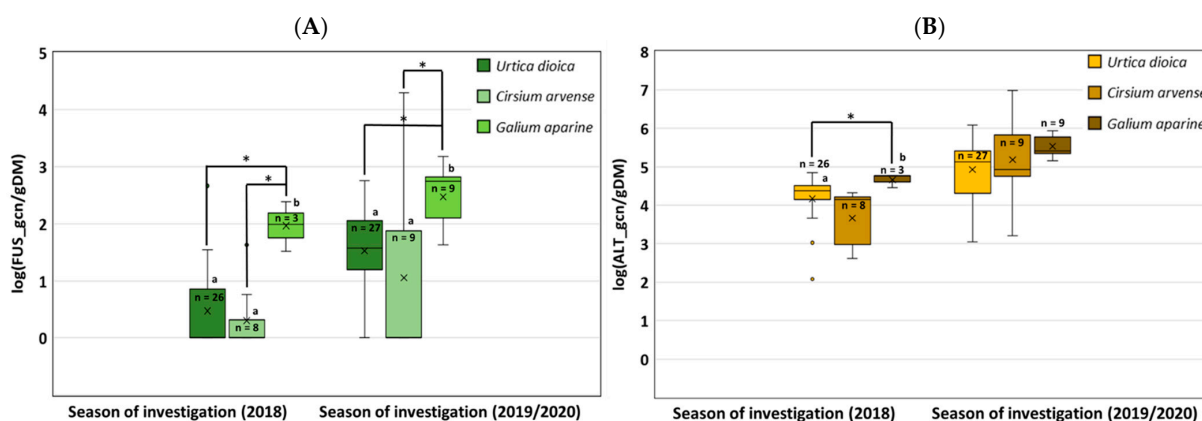


Figure 5. Abundance of *Fusarium* (A) and *Alternaria* (B) fungi expressed as LOG (x + 1) (gene copy numbers) of *Fusarium* (FUS_gcn/gDM) and *Alternaria* (ALT_gcn/gDM) on *Urtica dioica*, *Cirsium arvense* and *Galium aparine* in 2018 and 2019/2020. The different weed plants were sampled at the edges of all kettle holes investigated. The midline of the boxplots represents the median; x is the mean value; the upper and lower limits of the boxes are the third and first quartiles. Asterisk brackets indicate significant differences between parameters investigated (* *p*-values < 0.05). a and b highlight that there is a significant difference (*p* < 0.05) between the samples to be found.

Plants of the family of *Poaceae* were also divided into grasses and reed. No significant differences of *Fusarium* and *Alternaria* abundances were detected between these two plant species. However, in 2018 and in 2019/2020, grasses were trending as higher colonized

by *Fusarium* fungi compared to reed plants. In 2018, grasses were higher colonized by *Alternaria* fungi, and in 2019/2020, the mean values of *Alternaria* were even.

The results show a slight difference between grasses and reed in both seasons investigated. Thus, grasses have a higher fungal load than reed, and according to this, grasses are the most infected arable weed detected in our field study.

3.3. Community Composition of Different *Fusarium* Species Isolated from Arable Weeds (*Poaceae* and *Herbaceous Plants*)

Moreover, all arable weeds were analyzed to detect the diversity of all *Fusarium* species using them as host plants (Tables 3 and 4).

Table 3. *Fusarium* species isolated from all sampled plants at the edges of all kettle holes investigated in 2018. Sampling months are summarized. X stands for the presence of one specific *Fusarium* species (11 in total, column 1) on one plant species/family (line 1). Grasses are a mixture of the species *Arrhenatherum elatius*, *Bromus sterilis*, *Calamagrostis epigejos*, *Dactylis glomerata*, *Elymus repens*, *Lolium perenne*, *Poa trivialis* in different proportions.

2018	Grasses	<i>Urtica dioica</i>	<i>Cirsium arvense</i>	<i>Galium aparine</i>	<i>Phragmites australis</i>	<i>Sisymbrium loeselii</i>	<i>Arctium sp.</i>	Total Amount of Infected Weed Species
<i>F. arthrosporioides</i>	X	X			X		X	4
<i>F. avenaceum</i>	X	X			X			3
<i>F. cerealis</i>	X	X	X		X	X		5
<i>F. culmorum</i>		X		X	X			3
<i>F. equiseti</i>	X	X	X			X		4
<i>F. graminearum</i>		X	X				X	3
<i>F. oxysporum</i>				X				1
<i>F. poae</i>	X		X		X			3
<i>F. sambucinum</i>	X	X		X		X	X	5
<i>F. solani</i>	X							1
<i>F. sporotrichioides</i>	X	X	X					3
Unidentified species	X	X	X		X		X	5
Total amount of detected <i>Fusarium</i> species	8	8	5	3	5	3	3	

In total, 12 different *Fusarium* species were identified in this study over the two sampling years. Ten *Fusarium* species that occurred in the first year were also found in the second year (Tables 3 and 4); *F. solani* was only observed in 2018, whereas *F. tricinctum* occurred only in 2019/2020. Grasses belonging to the family of *Poaceae* were colonized by the highest number of different *Fusarium* species in both years, followed by *Urtica dioica* (Tables 3 and 4, Figure 6). In 2018, eight of eleven *Fusarium* species could be found on grasses, while in 2019/2020, grasses carried nine out of the eleven *Fusarium* species detected. The most frequently analyzed herbaceous plant *Urtica dioica* harbored between eight (2018) and seven (2019/2020) different *Fusarium* species in total. We were able to isolate fusaria from every plant species we investigated. In 2018, all analyzed plant samples were infested with at least three different *Fusarium* species. In 2019/2020, no plant species harbored less than four species. These results indicate that grasses not only harbor the highest amount of *Fusarium* and *Alternaria* in general, but are also infested with the most different species of *Fusarium* compared to other arable weeds, including reed.

Table 4. *Fusarium* species isolated from all sampled plants at the edges of all kettle holes investigated in 2019/2020. Sampling months are summarized. X stands for the presence of one specific *Fusarium* species (11 in total, column 1) on one plant species/family (line 1). Grasses are a mixture of the species *Arrhenatherum elatius*, *Bromus sterilis*, *Calamagrostis epigejos*, *Dactylis glomerata*, *Elymus repens*, *Lolium perenne*, *Poa trivialis* in different proportions.

2019/2020	Grasses	<i>Urtica dioica</i>	<i>Cirsium arvense</i>	<i>Galium aparine</i>	<i>Phragmites australis</i>	<i>Rumex sp.</i>	<i>Tanacetum vulgare</i>	Total Amount of Infected Weed Species
<i>F. arthrosporioides</i>		X						1
<i>F. avenaceum</i>	X	X	X	X	X	X	X	7 ¹
<i>F. cerealis</i>	X	X	X	X		X		5
<i>F. culmorum</i>	X	X						2
<i>F. equiseti</i>	X	X	X	X	X	X	X	7 ¹
<i>F. graminearum</i>	X	X	X	X	X	X	X	7 ¹
<i>F. oxysporum</i>							X	1
<i>F. poae</i>	X			X		X		3
<i>F. sambucinum</i>	X		X	X				3
<i>F. sporotrichioides</i>	X	X	X	X	X	X	X	7 ¹
<i>F. tricinctum</i>	X		X					2
Unidentified species	X	X						2
Total amount of detected <i>Fusarium</i> species	9	7	7	7	4	6	5	

¹ *Fusarium* species detected on every plant sample.

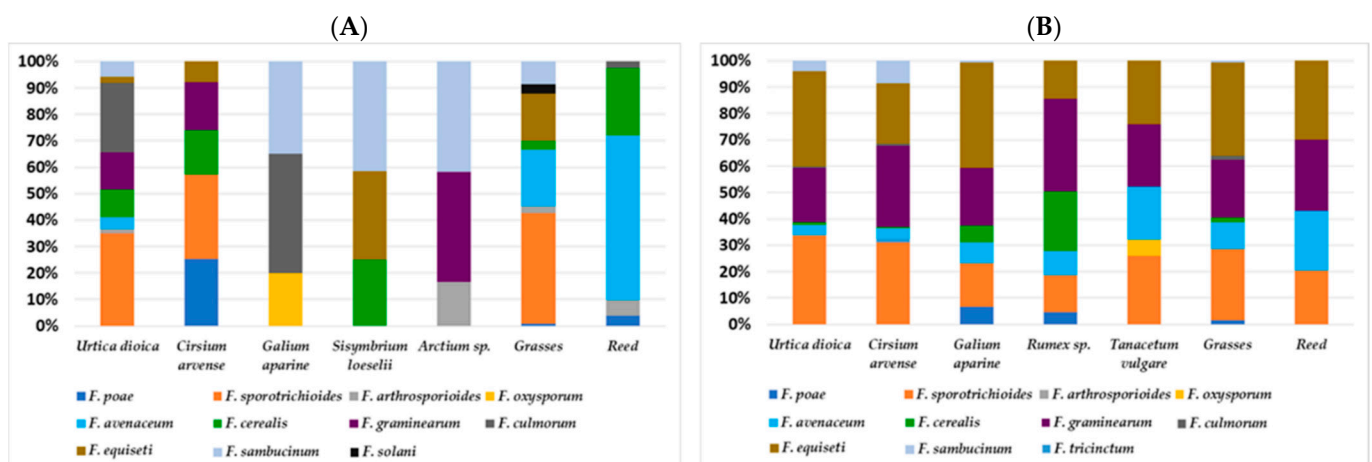


Figure 6. Quantitative community composition of *Fusarium* (F.) species (in %) based on the *Fusarium* abundances (FUS_cfu/gFM) of all different arable weeds analyzed in 2018 (A) and 2019/2020 (B) summarized over all sampling dates in autumn/winter and all edges of kettle holes ($n = 9$ /year) investigated in each season.

However, the abundances (FUS_cfu/gFM) of the different *Fusarium* species found each year differed remarkably. In 2018, the most frequently isolated species was *F. sporotrichioides* (21%), followed by *F. avenaceum* (14%) and *F. sambucinum* (8%). Less commonly detected species included *F. equiseti* (7%), *F. cerealis* (7%), *F. culmorum* (7%), *F. graminearum* (4%), *F. arthrosporioides* (2%), *F. poae* (2%), *F. solani* (1%), and *F. oxysporum* (1%) (Figure 6). Unidentified species comprised 26% (Table 3).

In 2019/2020, *F. equiseti* (35%) was the dominant species (Figure 6), while the predominant species were *F. sporotrichioides* (28%), *F. graminearum* (22%), and *F. avenaceum* (8%). Less commonly detected species included *F. sambucinum* (2%), *F. cerealis* (2%), *F. poae* (1%), *F. culmorum*, *F. tricinctum* (<1%), *F. oxysporum* (<1%), and *F. arthrosporioides* (Figure 6).

One percent of the isolated species could not be identified. *F. avenaceum*, *F. graminearum* and *F. equiseti* were the most widespread *Fusarium* species and were found on all seven sampled weed species 2019/2020 (Table 4).

In 2019/2020, the species composition of *Fusarium* on the different plant species was more balanced than in 2018. Four *Fusarium* species were found on every plant species investigated in 2019/2020 (Figure 6B), while in 2018, *F. sporotrichioides* was only detected on *Urtica dioica*, *Cirsium arvense* and on grasses. *F. equiseti* and *F. graminearum* were isolated from four plant samples, and *F. avenaceum* was found on three plants in 2018 (Figure 6A).

3.4. The Abundance of Plants of the Family of Poaceae and Herbaceous Plants at the Edges of Kettle Holes

By the previous results, it was shown that plants of the family *Poaceae* at the edges of kettle holes in autumn/winter harbor on average more fungi than other arable weeds. To show the importance of these plants for the habitat of kettle holes and as a source of infection with phytopathogenic fungi, the marginal vegetation (3 m from the field border into the kettle hole) was analyzed for the occurrence of plants belonging either to the family of *Poaceae*, to herbaceous plants or to woody plants.

At the edges of three out of six kettle holes, the quantity of plants of the family *Poaceae* was higher than the quantity of herbaceous plants (Figure 7). The number of plants belonging to the family of *Poaceae* varied from a minimum of 18% to a maximum of 65% plants at the edges. No kettle hole edge without vegetation of the family of *Poaceae* was identified. Herbaceous plants as well act as an attractive reservoir for phytopathogenic fungi of the genera *Fusarium* and *Alternaria* and were detected in high abundances from 23% up to 79% per kettle hole (Table 5).

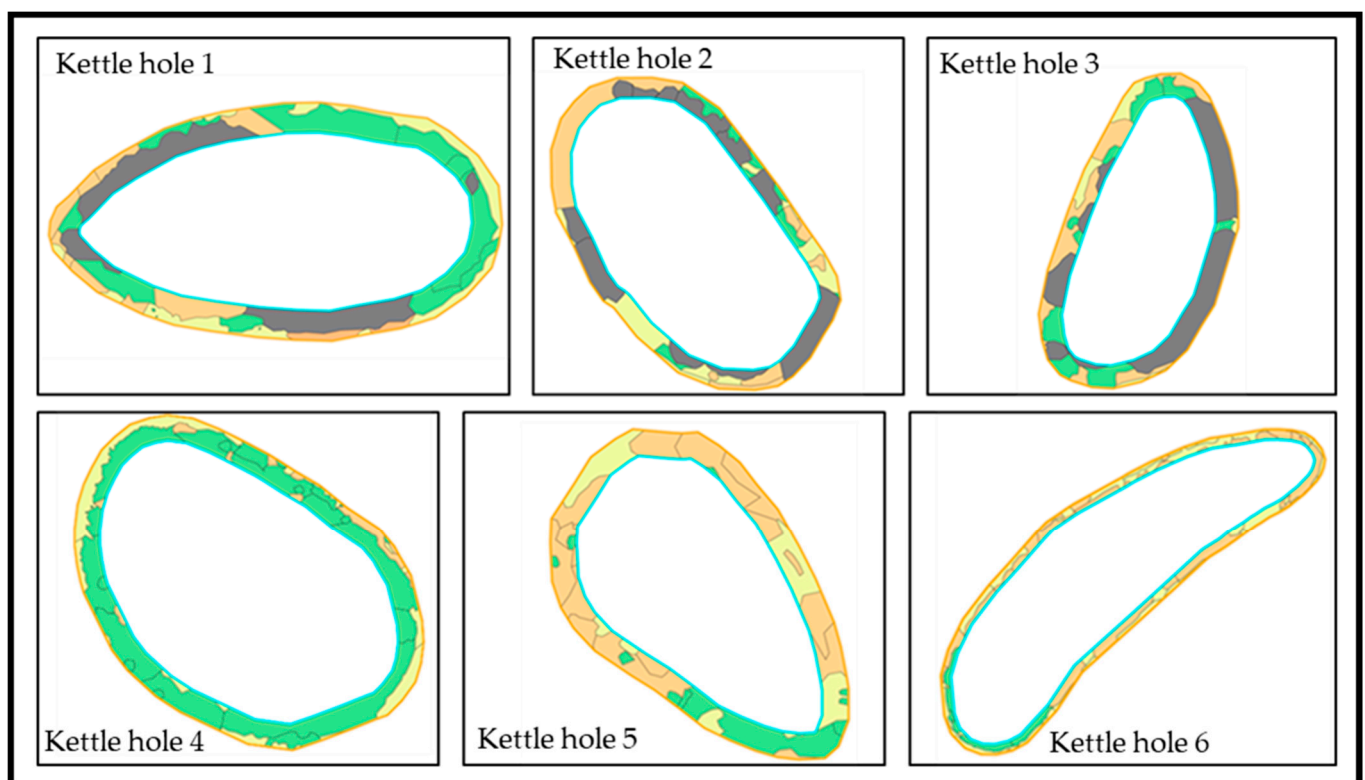


Figure 7. Digitalized vegetation at the edges of six different kettles holes investigated in 2019/2020 (exemplary). Different plant species were digitalized based on orthomosaics via manually mapping in QGIS. Green color, herbaceous plants; yellow color, *Poaceae*; orange color, mixture of herbaceous plants/*Poaceae*; grey color, woody plants.

Table 5. The amount of plants (*Poaceae*, herbaceous plants, woody plants) at the edges of six different kettle holes in m² and % sampled in 2019/2020. Vegetation at kettle holes edges was analyzed and digitalized in June 2020 by manual mapping in QGIS, based on orthomosaics.

Kettle Hole	<i>Poaceae</i> (m ²)	<i>Poaceae</i> (%)	Herbaceous Plants (m ²)	Herbaceous Plants (%)	Wood (m ²)	Wood (%)
1	76	25	117	38	113	37
2	125	33	88	23	169	44
3	53	18	90	31	149	51
4	76	22	271	78	0	0
5	161	52	147	48	0	0
6	591	65	320	35	0	0

4. Discussion

Arable weeds are part of every agricultural ecosystem. In addition to their ability to compete for vital resources including water, solar radiation and nutrients among others with the crops [42], they also play an important role in the life cycle of phytopathogenic fungi with wide host ranges [43]. In addition to being pest themselves, many weeds serve as alternative and alternate host plants for fungi and thus can help the pathogen to survive, especially during unfavorable environmental conditions or between two field seasons, when the main host is not available [43–46]. In case of non-crop hosts, Kumar et al. [43] differentiated between alternate (replacement for the original) and alternative (another option to the original) hosts. Our investigations took place in two consecutive autumn/winter seasons in 2018 and 2019/2020 when the fields were not cultivated. Thus, the non-crop plants investigated in our study were alternate hosts for pathogenic fungi and could help them survive the absence of their original hosts (wheat in our case). We focused on the suitability of these alternate hosts for fungi of the genera *Fusarium* and *Alternaria*, due to their special pathogenicity against (winter) wheat.

There are different sources of inoculum for fungal infections described in several studies [12,20]. These studies observed that residues from the previous crops were the primary source of inoculum fungal diseases, because contaminated crop debris from the last season can act as a reservoir for fungal pathogens. Moreover, many different weed species have been identified as non-crop hosts for *Fusarium* spp. [10,21,22,47–50] and thus can be taken into consideration as an overwintering habitat and source of infection for the next season's crop.

A study by Pereyra and Dill-Macky [20] showed that *F. graminearum* uses the graminaceous weeds *Lolium*, *Digitaria*, *Setaria*, and *Cynodon* as alternate hosts in winter. Other studies analyzed non-crop hosts growing simultaneously with crop hosts. The removal of these weeds could be used to avoid the transfer of pathogens from one season to another [46,51]. In our study, we focused on weeds growing in semi-natural habitats (kettle holes) inside the field. Semi-natural landscape elements offer different ecosystem services, e.g., an increase in biodiversity [52], but their existence in fields often poses challenges to farmers [53]. The weeds were not harvested alongside the crops, and they survive there for several seasons. The aim of this study in general was to determine the ability of these weeds to act as an overwintering habitat for pathogenic fungi and therefore to assess the hazard of the semi-natural landscape element kettle hole in arable lands to infect crops on the surrounding fields. In our field study, we analyzed seven typical German arable weeds, a mixture of seven grass species, and *Phragmites australis*, which is not an arable weed in general, but which is frequent on agricultural fields in Brandenburg due to its occurrence at the edges of small water bodies. The named plants were frequent in cultivated and non-cultivated landscapes in Brandenburg and were therefore chosen for the determination of their suitability as overwintering habitats for *Fusarium* and *Alternaria*. With our analyses, we were able to find out that all the weeds investigated in the present study harbored fungi in the autumn/winter seasons, including pathogenic fungi of the genera *Fusarium* and *Alternaria* in different abundances. Our results confirm previous studies that weeds and

grasses can be a reservoir for fungal pathogens. We can extend to the existing knowledge by eight central European plants as hosts for both *Fusarium* and *Alternaria* at the same time, in German agricultural landscapes. While we isolated 33% *Fusarium* from herbaceous plants in 2018 and 28% in 2019/2020, we found 67% for 2018 and 72% for 2019/2020 from gramineous hosts, especially grasses. Concerning *Alternaria*, in 2018, we found 37% of them on weeds and 63% on gramineous hosts. In 2019/2020, 34% of *Alternaria* was found on weeds, with 66% on gramineous hosts. This clearly shows that grasses are the most harmful weed in case of infection of the crops for the next season. Studies by Dong et al. [54] and Walsh et al. [55] further demonstrated that gramineous weeds were alternative hosts for different *Fusarium* species and that wild grasses harbor a high diversity of *Fusarium* species. Purs [56] found perithecia and sporodochia on senesced grasses collected in spring and therefore assumes that phytopathogenic fungi may also use wild grasses residues as non-crop hosts to overwinter. Costa et al. [57] noted that over the last years, “the interest in investigating *Fusarium* species associated with asymptomatic native or introduced plants has increased”. However, little is known about the importance of weeds in the *Fusarium* head blight disease cycle [21,58–60]. Thus, a better understanding of non-crop hosts of fungal pathogens is key to better describe their ecology and epidemiology [58].

As Kumar et al. [43] already mentioned, plant disease development is influenced by the host, the pathogens, and their interactions with the environmental driving factors. Many studies name environmental conditions, mainly temperature and relative humidity, as essential for the development of infection with *Fusarium* or *Alternaria* and distribution of an inoculum [10,26,61–64]. Others have reported that the monthly temperature, elevation, and other climate parameters also affect the distribution of *Fusarium* spp. [61,65–67]. In addition, environmental conditions had a greater influence on the survival of pathogenic fungi than the survival through chlamydospores [68].

In comparison to our knowledge on *Fusarium* fungi on non-host plants, there is a lack of information about the infection of arable weeds with species of the genera *Alternaria*, although the abundance of *Alternaria* is higher than the abundance of other fungi [69]. In this particular study, we analyzed the abundance of both genera to detect that *Alternaria* is also frequently found on arable weeds. We observed high abundances of *Alternaria* in both seasons. The ubiquitous genus of *Alternaria* includes pathogenic, endophytic and saprophytic species, and grows even at low temperatures [1,4,8].

Fusarium and *Alternaria* are often found together on plants when they share the same host. Then, they compete for the same resources [8]. The high abundances of *Alternaria* detected on the arable weeds in autumn and winter seasons in our study may result from the fact that *Alternaria* is more adapted to low temperatures and often acts as a saprophyte [4,8]. Most species of the genera *Fusarium* are mainly known as plant pathogens, while some are also considered saprophytes, commonly found in soil, on the grain surface and on dead plant residues [70,71]. These species are hemitrophs and are thereby able to change their lifecycle on the host plant from biotroph to necrotroph [21]. The results of Phan et al. [72] show that on non-crop hosts, *Fusarium* species can also be endophytes. Subsequent saprophytic infestation of weeds by *Fusarium* might also result in a reservoir of inocula in weed residues during overwintering [20]. As described above, in the autumn/winter seasons with low temperatures and considerable necrotic plant material, we detected high abundances of *Alternaria* on arable weeds at kettle holes in agricultural fields. Moreover, we also found fungi of the genera *Fusarium* in the cold seasons on partly senescent plant tissue.

The species composition can also be affected by differences in the saprotrophic capacity of different *Fusarium* species. *F. solani*, *F. oxysporum*, *F. poae*, and *F. sporotrichioides* are known to have a better saprotrophic capacity in crop residues and soil than *F. graminearum* [12,20]. Additionally, species from the *F. incarnatum-equiseti* species complex are considered to be saprophytes or pathogens [73]. We discovered high abundances of *F. sporotrichioides* in both autumn/winter seasons (21% in 2018; 28% in 2019/2020) that could be caused by a better saprotrophic capacity of *F. sporotrichioides*, including in weed residues. However,

in contrast to the findings of Leplat et al. [12], *F. solani*, *F. oxysporum*, and *F. poae* were not found frequently in 2018 and 2019/2020. *F. graminearum* was found in both seasons, but was more frequent in 2019/2020 (22%). We also isolated *F. equiseti* (2018: 7%, 2019/2020: 35%) and *F. avenaceum* (2018: 14% and 2019/2020: 8%) in higher abundances than the others. Maybe the saprotrophic capacity on weed residues differs to the capacity on crop residues, and/or the capacity depends strongly on precipitation.

This leads to the assumption that different *Fusarium* species are able to act hemitrophically on arable weeds in winter in Brandenburg. We observed this behavior for 12 *Fusarium* species in the two seasons investigated: *F. arthrosporioides*, *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. poae*, *F. sambucinum*, *F. solani*, *F. sporotrichioides* and *F. tricinctum*.

Most phytopathogens are able to infect a wide host range over the same or different family [74], and weeds associated with crops often come from the same family as the crop because they share several botanical similarities [43,74]. Landschoot et al. [10] mentioned that most *Fusarium* species have a wide host range among gramineous weeds, and Mourellos et al. [48] isolated *F. graminearum* from different healthy gramineous and non-gramineous weed species. The results of a study from Inch and Gilbert [50] indicated that wild grasses might be important in the survival of *F. graminearum*, *F. sporotrichioides*, *F. equiseti*, *F. avenaceum*, *F. poae*, *F. oxysporum*, and *F. culmorum*. This is consistent with the results we obtained in our study. Additionally, we isolated *F. arthrosporioides*, *F. cerealis*, *F. sambucinum*, *F. solani*, and *F. tricinctum* on wild grasses at kettle hole edges. On these grasses, we observed eight different *Fusarium* species in 2018 and nine different species in 2019/2020, the maximum of *Fusarium* species in one plant family. Suproniene et al. [22] also detected a maximum of nine *Fusarium* species on one plant.

Fusarium species are known to have different spore types. *F. graminearum* and *F. avenaceum*, for example, produce ascospores and micro- and macroconidia, while other species such as *F. culmorum* do not produce ascospores [6,75]. Although the ascospores were not specifically evaluated in this study, we assumed that the habitat at the kettle hole edges may support the production of ascospores. This spore type may also travel longer distances [75], which may account for the higher abundance of *F. graminearum* [76]. In accordance with the state of art, *F. graminearum*, *F. avenaceum* and *F. equiseti* were observed in high abundances in both seasons.

With our methods, we were able to detect 12 different *Fusarium* species on arable weeds in the autumn/winter seasons in 2018 and 2019/2020. *F. sporotrichioides* was the most frequently isolated species in 2018, but was only found in and on three weeds (grasses, *Urtica dioica*, and *Cirsium arvense*). *Galium aparine* was the most infested plant species both in 2018 and in 2019/2020, but only harbored three *Fusarium* species in 2018: *F. sambucinum*, *F. culmorum*, and *F. oxysporum*, while the last two were not among the dominant species this season.

In 2019/2020, we generally found that more *Fusarium*. *F. equiseti*, *F. sporotrichioides*, *F. graminearum*, and *F. avenaceum* were detected on every plant species investigated. On *Galium aparine*, we found seven different *Fusarium* species, including *F. graminearum*, *F. equiseti*, *F. sporotrichioides* and *F. avenaceum*. In addition, less common species in 2019/2020 such as *F. cerealis*, *F. sambucinum* and *F. poae* were detected on this plant species. Maybe the special sticky texture of the leaves is able to catch many different spore types and forms, even when they were not as abundant as other species.

In 1984, Helbig and Carroll [77] sampled different weed plants from soybean fields in the United States of America and isolated *Fusarium oxysporum* from 16 different weed species. This result shows that weeds can serve hosts for this species. In 2008–2009, fresh weeds and weed residues from 12 weed families were analyzed in Croatia, isolating 14 different *Fusarium* species, with *F. graminearum* as the most frequently isolated one [47]. Suproniene et al. [22] detected that weed species in cereal-based crop rotations were infested by *Fusarium*. Twenty-seven species were identified as new hosts for fungi of the genera *Fusarium*. This is consistent with the results we obtained in our study. Martínez

et al. [58] isolated a high diversity of *Fusarium* species from only a small area of arable land (one hectare). These results show great species richness and can be confirmed by our findings. We also took samples from a small area of wheat fields (1 × 1 m/kettle hole edge) and were able to isolate 12 different *Fusarium* species from the weeds growing there.

In 2006 and 2007, Görtz et al. [78] isolated 13 different *Fusarium* species from maize samples. In the first year, *F. verticillioides* was the dominant species, followed by *F. graminearum*, *F. proliferatum*, and *F. equiseti*. In addition, *F. cerealis*, *F. avenaceum*, *F. sporotrichioides*, *F. oxysporum*, *F. poae*, *F. subglutinans*, *F. venenatum* and *F. tricinctum* were isolated in this study, but in lower abundances. In 2007, *F. graminearum* was the most dominant species, followed by *F. cerealis*, *F. subglutinans*, and *F. avenaceum*. As well, *F. verticillioides*, *F. proliferatum*, *F. equiseti*, *F. culmorum*, *F. tricinctum*, *F. poae*, and *F. sporotrichioides* were isolated less often.

Fallahi et al. [79] investigated different *Fusarium* species on maize in 2015 and 2016 in Iran. The main isolated species were *F. verticillioides* and *F. proliferatum*. In lower abundances, they also found species belonging to the *F. incarnatum equiseti* species complex, *F. thapsinum* and *F. redolens*. In a study carried out in 2017 and 2018 in Flanders, Belgium, *F. verticillioides* was the most detected *Fusarium* species on maize samples. *F. graminearum* and *F. culmorum* were also found in high abundances [80]. Pfordt et al. [81] isolated *F. graminearum*, *F. verticillioides*, and *F. temperatum* from maize ears, while again *F. graminearum*, but also *F. equiseti*, *F. culmorum*, and *F. temperatum* were more often detected on maize stalks. The differences in the species composition on maize plants (ears and stalks) are mainly caused by weather variations between the years and the microclimate at the different locations [78,81].

As maize was the preceding crop in our study, the species composition on our weed plant samples might also have been influenced by the *Fusarium* species overwintering on maize stubble remaining in the fields. During our investigations, we did not isolate *F. verticillioides* or *F. proliferatum*, which were two of the most isolated species from maize. However, *F. graminearum* is a causal agent of maize diseases, which we also found in high abundances at the kettle holes, especially in 2019/2020. We also frequently detected *F. equiseti*. The high abundances of this species isolated in our study could be associated with leftover maize stalks on the fields investigated. Pfordt et al. [81] mentioned that *F. equiseti* is often found on maize samples, mainly on the stalks, which are more often found as crop residues than maize ears.

The community composition of *Fusarium* fungi could depend on many factors, including the study side, the current and the preceding crops, the weeds, and the climate condition. Regional differences in species profile may exist due to different crop rotation systems and local climatic conditions (especially temperature and precipitation).

However, it seems to be confirmed that weeds offer a habitat for the majority of *Fusarium* species. To the best of our knowledge, this is the first time that *Urtica dioica*, *Cirsium arvense*, *Galium aparine*, *Phragmites australis*, *Sisymbrium loeselii*, *Arctium* sp., *Tanacetum vulgare*, and *Rumex* sp. are reported as inoculum reservoirs of *Fusarium* in agricultural landscapes in Brandenburg, Germany. Furthermore, we were able to observe that they also offer a habitat for *Alternaria*, even in higher amounts.

The hazard of infection by weeds growing in the vicinity of crops in arable lands has increased lately through less use of tillage and chemical herbicides. A significant increase in conservation tillage practices in the last decade was noted by Dong et al. [54], McMullen et al. [82], Manghwar et al. [83], leading to greater amounts of last-season crop residues, and therefore inocula for fungal infections on the soil surface. The authors [54,82,83] analyzed that *F. graminearum* in soil residues was found more abundantly in areas where minimum tillage practices were used, although a study by Lori et al. [84] showed that *Fusarium* produces less spores on weeds than on crop residues. Specific management practices could increase the survival of pathogenic fungi in weeds, including no-tillage practices, an inappropriate crop rotation (e.g., wheat/wheat), and enhanced use of herbicides. The latter promotes the selection and survival of resistant weeds as well as changes the abundance and richness of weeds [22,58]. In China, 70% of gramineous weed species was resistant to herbicides, which makes it hard to control and a good habitat for

Fusarium species [54]. Due to the ban on the use of chemical applications next to kettle holes, arable weeds at their edges can grow undisturbed. Farmers cannot control the growth of the weeds with herbicides, nor the infestation of fungal pathogens with fungicides.

Additionally, weeds in general are often more genetically diverse than cultivated plants, and are therefore less susceptible to diseases themselves [43]. Linde et al. [74] also pointed out that pathogens from monocultural hosts may be less virulent than pathogens from genetically diverse hosts. Field sanitation, the removal and destruction of crop residues, and non-crop hosts can reduce the transmission of pathogens from one season to another [45,48,54]. Since alternative and alternate hosts play an essential role in spatial and temporal distribution of fungi, knowing the weed species that harbor fungal pathogens can help in the implementation of more effective management practices [58,85]. In the case of analyzing non-crop host plants as inocula for fungal infections, our study contributes to the identification eight new alternate host plants for both *Fusarium* and *Alternaria*. Regarding the risk of a serious infection of cereal plants, weeds at the edges of kettle holes in Brandenburg harbor these phytopathogenic genera and species.

Especially, the fact that many non-crop hosts are symptomless while harboring the fungal pathogen shows that the risk originating from them might be underestimated, which makes the management more challenging [85–87]. In general, the contribution of weeds to the infection of crops with phytopathogenic fungi should be considered in pest management strategies because they offer a great habitat, especially under favorable weather conditions or in the absence of original host plants. Additionally, plants growing in semi-natural landscape elements such as kettle holes should be regarded as a special hazard due to the presence of the weeds over the entire year.

5. Conclusions

Arable weeds, as part of every agricultural ecosystem, should be taken into account as a source of inocula for fungal infections. Pathogenic fungi use these plants as hosts, especially when their main host is absent. If possible, the removal of weeds is necessary in order to prevent the transmission of pathogenic fungi. Regarding kettle holes, farmers may be encouraged to avoid high grass stripes around the kettle holes in their fields. If they mow the edges of kettle holes, we recommend disposing of the plant material. In our study, we detected eight central European plants as new non-crop host plants for *Fusarium* and *Alternaria* in Brandenburg, Germany. Since the hazard of infection by arable weeds has increased lately through less use of tillage and chemical herbicides, more importance should be given to the detection of further (potential) non-crop host plants for *Fusarium* and *Alternaria*. Especially due to the fact that many non-crop hosts are symptomless while harboring the fungal pathogens, the risk originating from them might be underestimated and makes management more challenging. For this reason, future work will need to aim to identify further non-crop host plants and reasons for the susceptibility of different weed species to fungal infections.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy12040823/s1>, Table S1: Primers used for the qPCR assay of *Fusarium* and *Alternaria*.

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