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Effect of Different Doses of Ash from Biomass Combustion on the Development of Diatom Assemblages on Podzolic Soil under Oilseed Rape Cultivation

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Abstract: The aim of the study was to determine the effect of fertilization with various doses of ash from biomass combustion (balanced to the amount of K_2O introduced into the soil) on the growth of representatives of soil microorganisms, i.e., diatoms. In a one-factor field experiment (control, NPK, 100, 200, 300, 400, 500 kg/ha), soil samples were collected from the 0–5 cm layer of podzolic soil under the cultivation of winter oilseed rape (*Brassica napus* L. var. *napus*) at the end of August 2019 and 2020. The biomass combustion ash used for soil fertilization was characterized by an alkaline reaction (pH = 12.83 ± 0.68) and high levels of basic macroelements required for proper plant growth and development. The particle size distribution in each plot was identified as loamy silt (pgl). Before the experiment (autumn 2018), the soil exhibited an acidic reaction (pH_{H_2O} = 5.8), low conductivity (EC = 68 µS), and 19.09% moisture at the 0–5 cm level. In total, 23 diatom species were identified in the material collected from the topsoil in all variants of the experiment. *Hantzschia amphioxys*, *Mayamaea atomus*, *Mayamaea permitis*, *Nitzschia pusilla*, *Pinnularia obscura*, *Pinnularia schoenfelderi*, and *Stauroneis thermicola* were the most abundant populations.

Keywords: biomass ashes; terrestrial diatoms; fertilization; algae; field experiment

Citation: Stanek-Tarkowska, J.; Szostek, M.; Rybak, M. Effect of Different Doses of Ash from Biomass Combustion on the Development of Diatom Assemblages on Podzolic Soil under Oilseed Rape Cultivation. *Agronomy* **2021**, *11*, 2422. https://doi.org/10.3390/agronomy11122422

Academic Editor: Francesco Montemurro

Received: 16 November 2021
Accepted: 22 November 2021
Published: 27 November 2021

1. Introduction

The growing use and processing of wood has led to the generation of waste products, e.g., woodchips used for energy production, and increased production of wood ash [1,2]. Wood ash is often regarded as a waste product. It retains most of the main minerals necessary for plant nutrition, except nitrogen, and has liming properties due to the high content of metal oxides and hydroxides [3,4]. Ashes are a source of plant nutrients (macro- and microelements) and can be regarded as a substitute for calcium fertilizers. Their deacidifying properties are associated with their high concentrations of calcium and magnesium carbonate [5–7]. Zając [7] determined the content of valuable nutrients in ash from biomass combustion. As indicated by previous research [8–11], higher doses of ash may exert a deacidifying effect and improve the physicochemical properties of light soils. However, a high ash content does not always indicate effective deacidification, which depends on many factors, e.g., ash properties (granularity, hydration, particle size distribution, water solubility, etc.) and the buffering capacity of soils. The addition of ashes into soils requires careful determination of their optimal dose to avoid damage to the biological soil structure. Importantly, biomass ashes may contain substantial amounts of trace elements and heavy metals, which are not always desirable in the soil environment [7].

Soil ecosystems are characterized by unstable environmental conditions. Large fluctuations in moisture and temperature, as well as human activity such as plowing at high soil moisture levels or the use of fertilizers and plant protection agents, may exert an adverse effect. The maintenance of biodiversity is essential for the sustenance of the ecological functions and processes that ensure soil fertility and productivity of agricultural ecosystems. One of the indicators of biodiversity is the presence of both prokaryotic and eukaryotic soil algae, which constitute a very important group of soil microorganisms. Most algae are cosmopolitan organisms; they are mostly represented by the following: Green algae, cyanobacteria, diatoms, xanthophytes, and, less frequently, euglenids or red algae [12–22]. Soil algae are the primary producers of organic substances and are a source of food for heterotrophic soil organisms.

Similarly to aquatic diatom species, terrestrial diatoms are sensitive to many environmental factors, e.g., pH, anthropic disturbances, soil moisture, and biogen concentrations. However, in contrast to the autecological preferences of aquatic diatoms for several important variables, such preferences of many terrestrial diatom species are not well known. This suggests that their potential as biological indicators is still poorly understood [23]. So far, there have only been a few investigations involving a sufficiently large number of samples to determine the ecological preferences of individual species [23–25].

The aim of the present study was to determine the impact of short-term fertilization with biomass combustion ashes on diatom assemblages present on the soil surface.

2. Materials and Methods

The field experiment was established in autumn 2018 in Korzenica (Podkarpackie Province, Jarosław County, GPS coordinates: 500.02′.16.3N, 220.55′.06.4E). The experiment was set up in the arable layer (0–27 cm) of podzolic soil with the particle size distribution of loamy silt (Working Group WBR 2006). The one-factor experiment consisted of cultivation of winter oilseed rape (*Brassica napus* L. var. *napus*), cultivar Mandril (Syngenta). In this field experiment, a compact strip was divided into 7 sub-blocks, each with an area of 162 m^2, where different fertilization variants were applied. Each variant in the experiment was applied in three replicates. The fieldwork was started in the autumn of 2018. A conventional mineral fertilizer NPK and an unconventional fertilizer, i.e., ash from combustion of biomass (willow *Salix viminalis* L.), were used in the experiment. The biomass ash doses were balanced to the amount of potassium introduced into the soil. In all experiment variants, constant nitrogen (81.3 kg N ha^{-1}) and phosphorus (34 kg P ha^{-1}) mineral fertilization were applied.

The fertilization of the winter rape plants at the following levels was the experimental factor:

- Control—no K_2O fertilization;
- NPK K_2O in mineral fertilizers (127 kg K_2O ha^{-1});
- 100 kg K_2O ha^{-1} in ash (0.5 t ha^{-1} of ash in bulk weight);
- 200 kg K_2O ha^{-1} in ash (1.0 t ha^{-1} of ash in bulk weight);
- 300 kg K_2O ha^{-1} in ash (1.5 t ha^{-1} of ash in bulk weight);
- 400 kg K_2O ha^{-1} in ash (2.0 t ha^{-1} of ash in bulk weight);
- 500 kg K_2O ha^{-1} in ash (2.5 t ha^{-1} of ash in bulk weight).

The fertilization was applied before sowing. The doses and dates of application are presented in Table 1.

Composition of biomass ash used in the experiment for fertilization of winter oilseed rape on podzolic soil was presented in Table 2.

Table 1. Fertilizers used in the field experiment in 2018–2021.

Fertilizer-Trade Name	Amount of Pure Component in 100 kg of the Fertilizer	Dose (kg/L per 1 ha)		Date of Application
		Fertilizer	Pure Component	
Biomass combustion ash	1.63 %P (3,73 kg P), 19.4% K (23.37 kg K), 4.96% Mg (8.222 kg Mg)	Varied depending on the experimental variant		30 August 2018 29 August 2019 25 August 2020
Monoammonium phosphate (MAP) $NH_4H_2PO_4$ (12%N-NH_4, 52% P_2O_5, 22.7% P)	22.7 kg P	150	34	30 August 2018 (all variants) 29 August 2019 (all variants) 25 August 2020 (all variants)
	12 kg N		18	
Potassium salt (60%)	60 kg K	175	105	30 August 2018 (NPK variant only) 29 August 2019 (NPK variant only) 28 August 2020 (NPK variant only)
RSM ® 32% N (aqueous solution of urea-ammonium nitrate, density 1.32 kg/dcm^3)	42.2 kg N (32 × 1.32)	150	63.3	4 March 2019 10 March 2020 15 March 2021

Table 2. Composition of biomass ash used in the experiment for fertilization of winter oilseed rape on podzolic soil.

pH H_2O	EC μS·cm^{-1}	Ca (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})	P (mg kg^{-1})
12.82	8.81	145,081	129,617	1452	9244

Soil was sampled at a depth of 0–5 cm from the arable layer before the experiment in the autumn of 2018 and in each experimental year after plant harvest (at the end of August). The following physicochemical properties were determined in the soil samples after drying and sieving through a sieve with a 2 mm mesh diameter. Soil reaction was measured with the potentiometric method using an HI-4221 pH meter (Hanna Instruments, Nusfalau, Romania) at a soil-to-solution ratio of 1:2.5. Soil electrolytic conductivity (EC), which is a measure of soil salinity, was determined with the conductometric method using an HI-2316 EC meter (Hanna Instruments, Nusfalau, Romania) at a soil-to-solution ratio of 1:5. Soil moisture was measured with the drying-weighing method after soil sampling in Kopecki cylinders.

The impact of the biomass combustion ashes on the selected physicochemical properties of the podzolic soil was analyzed using STATISTICA 13.3 software (StatSoft, Tulsa OK Oklahoma, USA). One-way analysis of variance (ANOVA) was performed using the Tukey HSD multiple comparison test to identify homogeneous groups ($p < 0.05$).

Diatom Processing

Soil samples for diatom analysis were taken from a layer of 0–5 cm and transferred to Petri dishes. The samples were digested in a mixture of concentrated sulfuric acid (H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$) to obtain clean diatom frustules. To remove the sulfochromic mixture, the material was centrifuged at 2500 rpm with distilled water several times until the chromic mixture was completely removed. Excess inorganic matter within the soil was removed by sedimentation. Cleaned diatom valves were placed onto coverslips and left to dry. When the material was completely dry, the coverslips with diatoms were mounted in synthetic Pleurax resin, ZBE Kraków, Poland (refractive index: 1.75).

Diatoms were identified and counted under an LM Carl Zeiss Axio Imager A2 equipped with a 100× Plan Apochromatic objective with differential interference contrast (DIC) for oil immersion. Diatom images were captured with a Zeiss AxioCam ICc5 camera (Jena, Germany). They were counted to 400 valves by scanning transects across the coverslip. The identification of diatom species was based on studies conducted by Krammer [26], Hofmann et al. [14] and Levkov et al. [27].

3. Results and Discussion

The meteorological conditions prevailing during the experiment, i.e., the mean air temperature and precipitation totals are presented in Table 3. The total annual precipitation in 2019 was 34.3 mm lower than in 2020. In August, i.e., the month of collection of the soil samples, the total monthly precipitation was 14.8 mm in 2019 and only 7.3 mm in 2020. Such low precipitation rates exert a limiting effect on the growth of diatom communities; they prefer moist habitats and, as autotrophic organisms, colonize the soil surface, which is characterized by the quickest water loss.

Table 3. Weather conditions in 2018/2019 and 2020 provided by the Meteorological Station of the University of Rzeszów.

	Temperature in °C				Precipitation in mm			
				2018				
Month	Ten-Day Period			Mean	Ten-Day Period			Total
	I	II	III		I	II	III	
IX	17.5	17.0	15.3	16.6	60.6	7.9	15.3	83.8
X	9.6	11.2	8.9	8.4	4.8	0.0	34.8	39.6
XI	10.2	3.3	−1.5	4.0	1.4	10.6	4.9	16.9
XII	−0.3	−2.0	1.97	−0.13	16.7	7.7	24.5	48.9
				2019				
I	−4.4	−1.4	−3.7	−3.16	18.0	14.2	7.6	39.8
II	1.3	3.4	1.5	2.1	4.8	3.1	5.7	13.6
III	4.7	4.0	4.9	4.5	4.8	14.3	4.0	23.1
IV	8.6	7.0	13.3	9.6	2.7	2.5	48.3	5.5
V	9.2	14.2	16.1	13.1	31.6	32.0	50.3	113.9
VI	19.0	22.7	21.1	20.9	3.7	18.1	4.1	25.9
VII	17.1	16.0	21.5	18.2	5.2	18.1	25.3	48.6
VIII	22.2	18.6	20.3	20.37	1.2	0.0	6.3	14.8
IX	16.6	13.9	14.8	15.1	2.1	0.5	18.3	20.9
X	14.5	8.3	12	11.6	13.5	28.2	11.6	53.3
XI	13.1	9.1	10.2	10.8	21.5	32.6	24.2	78.3
XII	7.7	5.4	0.9	4.6	5.6	3.8	6.4	15.8
				Total				501.5
				2020				
I	0.5	2.3	1.4	1.4	3.9	0.1	7.9	11.8
II	2.7	4.7	4	3.8	23.7	8.2	21.5	53.3
III	5.3	7.2	2.9	5.1	15.0	2.9	2.0	19.8
IV	7.9	8.5	11.2	9.2	0.0	4.7	5.3	10.0
V	11.1	11.1	11.7	11.3	25.3	24.4	33.6	83.3
VI	15.9	19.0	19.5	18.1	20.2	22.6	120.0	162.9
VII	19.9	17.1	19.3	18.8	10.2	8.5	0.2	18.9
VIII	20.2	19.5	20.1	19.9	0.2	0.1	7.0	7.3
IX	15.7	14.7	14.5	15.0	4.2	0.0	39.3	43.5
X	13.8	8.3	11.1	11.1	16.8	30.4	7.1	54.3
XI	12.9	8.5	10.5	10.6	17.5	29.8	8.1	55.4
XII	7.5	6.4	1.0	5.0	7.4	2.3	5.6	15.3
				Total				535.8

3.1. Soil pH

Soil pH is one of the most important determinants of soil processes and the growth of micro- and macro-organisms. Before the experiment, the soil had an acidic pH of 5.83. Changes in the pH value in the 0–5 cm layer of the podzolic soil are shown in Figure 1. In both 2019 and 2020, the lowest pH values were recorded in the soil variant to which only NPK mineral fertilization was applied. In the ash-fertilized variants, the pH values did not differ significantly in 2019, whereas the highest pH values in 2020 were found in the variants treated with 200 and 300 kg K_2O/ha. Nevertheless, the application of biomass combustion ashes did not increase the reaction in any variant. It was also observed that the pH values were higher in the first year of the experiment, but declined in the following year in all the variants. The high pH value of ashes does not always correspond to their deacidification ability, which depends on many factors, e.g., ash properties (granularity, hydration, particle size distribution, water solubility, etc.) and soil buffering capacity.

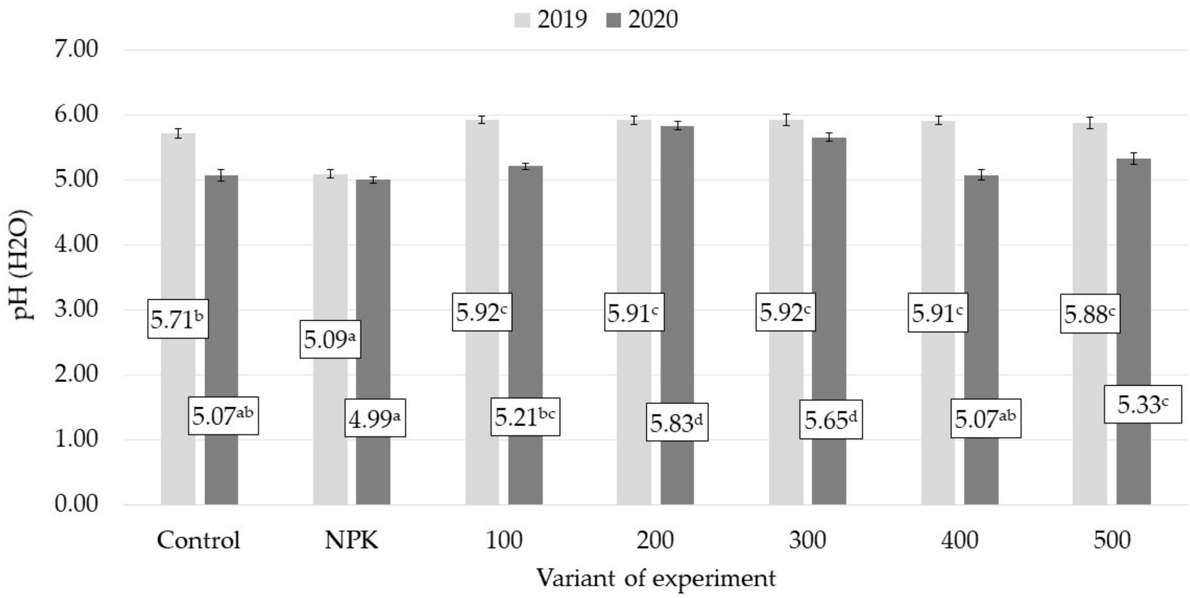

Figure 1. Changes in the pH value of the podzolic soil in the 0–5 cm layer after application of biomass combustion ashes (mean ± SD). Identical superscripts indicate that there are no significant ($p > 0.05$) differences between the experimental variants, as shown by the Tukey HSD post hoc test.

3.2. Salinity

The content of various salts in soil can be determined indirectly by measuring their content in the soil solution based on ionic conductivity, known as electrolytic conductivity (EC). The increase in salinity in the analyzed podzolic soil was induced by the application of both NPK mineral fertilization and biomass combustion ashes (Figure 2). In the first study year, the application of combustion ashes did not increase the salinity of the soil in the 0–5 cm layer. Except for the variant treated with 200 kg K_2O/ha of ash, the highest EC values were recorded in the control and the NPK-fertilized variant. A different tendency was observed in the second study year, as the application of ashes at a dose of 200 kg K_2O ha^{-1} and higher significantly increased the soil salinity value. Despite the increase in the mean EC values induced by NPK and ash fertilization, the salt concentration in the soil solution was within the range tolerated by all plant species.

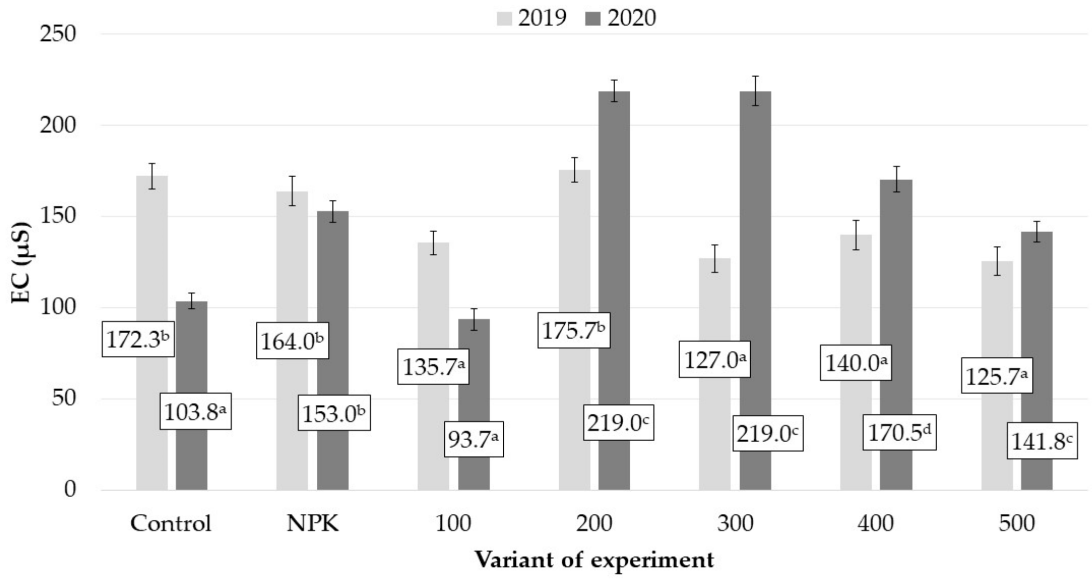

Figure 2. Changes in the salinity of the podzolic soil in the 0–5 cm layer after application of biomass combustion ashes (mean ± SD). Identical superscripts indicate that there are no significant ($p > 0.05$) differences between the experimental variants, as shown by the Tukey HSD post hoc test.

3.3. Soil Moisture

Soil moisture primarily depends on weather conditions, and, in particular, on the level of precipitation or hydration, and on the type of soil and agrotechnical treatments. As mentioned earlier, the experiment was conducted in the field, hence the close relationship with weather conditions. The years 2018, 2019, and 2020 were characterized by low total annual precipitation (Figure 3). August, i.e., the month when the samples were collected, was dry in both study years, as indicated by the percentage of soil water content. The various doses of ash fertilization did not increase the water retention capacity of the analyzed podzolic soil.

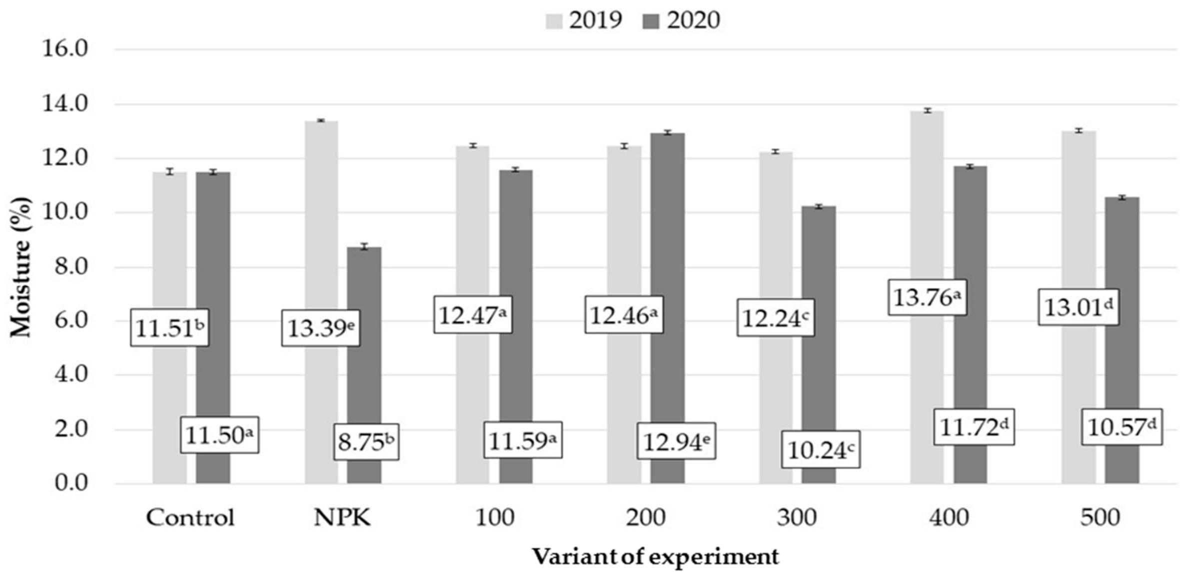

Figure 3. Changes in the moisture of the podzolic soil in the 0–5 cm layer after application of biomass combustion ashes (mean ± SD). Identical superscripts indicate that there are no significant ($p > 0.05$) differences between the experimental variants, as shown by the Tukey HSD post hoc test.

3.4. Terrestrial Diatoms

In the analyzed assemblages, the most numerous populations were formed by the following: *Hantzschia amphioxys* (up to 58%), *Mayamaea atomus* (up to 36.5%), *Mayamaea permitis* (up to 38.2%), *Nitzschia pusilla* (up to 27.9%), *Pinnularia obscura* (up to 39.7%), *Pinnularia schoenfelderi* (up to 15.6%), and *Stauroneis thermicola* (up to 41.8%).

Most of the 23 taxa of diatoms identified during the study represented a group of neutrophilic species (9 species), while the other group comprised species with still unknown preferences for a specific pH value (7 species) (Table 4, Figure 4). Acidophilic and alkaliphilic diatoms were represented by only a few species. However, the percentage share of the individual ecological groups in the analyzed assemblages was significantly different. The largest share in the assemblages was found for alkaliphilic and neutrophilic species, while acidophilic species and species with unknown preferences never exceeded 2%.

Currently, approximately 250 diatom taxa are considered as species with an ability to grow in terrestrial habitats. However, most studies of terrestrial diatoms are focused on areas of Europe and polar regions [23]. Therefore, it can be expected that the real number of terrestrial species is substantially higher, which seems to have been confirmed recently by taxonomic investigations covering both Europe [28–31] and tropical zones [32–37].

Table 4 shows the identified taxa with their pH preferences, as proposed by Van Dam et al. [38]. Importantly, Van Dam et al. [38] compiled these preferences of diatoms living in aquatic environments. No such classification has been proposed for terrestrial environments to date. A key issue is that species colonizing aquatic environments may exhibit different preferences in the soil environment. It can be assumed that diatoms have high adaptability to environmental conditions (reaction), as indicated by the present results. Taxa that prefer an alkaline environment, according to the classification proposed by Van Dam et al. [38], were found on slightly acidic soil. Nevertheless, *Mayamaea permitis*, *M. atomus*, *Hantzschia amphioxys*, *Pinnularia obscura*, and *Stauroneis thermicola* were the most frequent taxa in the community, which may indicate their considerably higher tolerance to environmental pH variation. Similarly, other studies [23] have confirmed that the optimum reaction value for the aforementioned taxa in terrestrial environments is slightly below pH 7. Of note, these taxa are some of the most frequently identified species in various natural and agricultural types of soil [20,39].

It is also worth emphasizing that two species of the genus *Microcostatus* (*M. aerophilus* and *M. dexterii*) were noted in the analyzed material. Both of these species have been described as new to science in recent years [22,31]; therefore, each report of their presence contributes to better understanding their ecological preferences and distribution.

Until recently, soil moisture was regarded as the main factor exerting an impact on communities of terrestrial diatoms. However, studies conducted by Foets [40,41] have shown that soil moisture does not influence the composition of the community, but is a key determinant of the absolute numbers of diatoms [42]. This was evidently confirmed by the number of diatoms identified in 2019, which was characterized by slightly higher soil moisture. In 2019, 18 taxa in the group representing the range from 15 to 30% were identified in the experimental variants, whereas 16 taxa were determined in 2020 at a lower moisture level. In their research, Foets et al. [23] have shown that diatoms grow well in terrestrial communities with at least a 20% soil moisture content. In the present field experiment, the maximum moisture content did not exceed 13.4%, due to the low precipitation rates in both years. The low soil moisture was probably the main cause of the low number of taxa (23) identified in this study. It seems that natural factors, such as rainfall and the humidity of agricultural soil, have a much greater impact on soil diatom assemblages than agricultural practices.

Table 4. List of identified taxa together with the percentage share in the assemblage and pH requirements (according to Van Dam et al., 1994). C—control (without fertilization), NPK K_2O in mineral fertilizers, 100–100 kg K_2O ha^{-1} in ash (0.5 t ha^{-1} of ash in bulk weight), 200–200 kg K_2O ha^{-1} in ash (1.0 t ha^{-1} of ash in bulk weight), 300−300 kg K_2O ha^{-1} in ash (1.5 t ha^{-1} of ash in bulk weight), 400−400 kg K_2O ha^{-1} in ash (2.0 t ha^{-1} of ash in bulk weight), 500−500 kg K_2O ha^{-1} in ash (2.5 t ha^{-1} of ash in bulk weight).

Taxa	2019							2020							pH Requirements
	C	NPK	100	200	300	400	500	C	NPK	100	200	300	400	500	
Hantzschia abundans Lange-Bertalot															neutrophilic
Hantzschia amphioxys (Ehrenberg) Grunow															neutrophilic
Humidophila contenta (Grunow) R.L.Lowe															alkaliphilic
Luticola aff. ***Acidoclinata*** Lange-Bertalot															unknown
Luticola pulchra (McCall) Levkov et al.															unknown
Luticola aff. ***Vesnae*** Levkov et al.															unknown
Mayamaea atomus (Kützing) Lange-Bertalot															alkaliphilic
Mayamaea fossalis (Krasske) Lange-Bertalot															neutrophilic
Mayamaea permitis (Hustedt) K.Bruder & Medlin															alkaliphilic
Microcostatus aerophilus Stanek-Tarkowska et al.															unknown
Microcostatus dexteri Stanek-Tarkowska et al.															unknown
Nitzschia frustulum (Kützing) Grunow															alkaliphilic
Nitzschia pusilla Grunow															neutrophilic
Pinnularia borealis Ehrenberg															neutrophilic

Table 4. *Cont.*

Taxa	2019							2020							pH Requirements
	C	NPK	100	200	300	400	500	C	NPK	100	200	300	400	500	
Pinnularia obscura Krasske	>15–30%	>5–15%	>15–30%	>15–30%	>0.1–5%	>5–15%	>5–15%	>30%	>5–15%	>5–15%	>5–15%	>5–15%	>5–15%	>5–15%	neutrophilic
Pinnularia schoenfelderi Krammer	<0.1%	<0.1%	>0.1–5%	>5–15%	>0.1–5%	>0.1–5%	<0.1%	>5–15%	>5–15%	>15–30%	>5–15%	>15–30%	>5–15%	>5–15%	neutrophilic
Pinnularia sinistra Krammer												<0.1%	<0.1%	>0.1–5%	acidophilic
Placoneis hambergii (Hustedt) Bruder												<0.1%	<0.1%		acidophilic
Sellaphora atomoides (Grunow) Wetzel et al.						>0.1–5%					<0.1%				unknown
Sellaphora nana (Hustedt) Lange-Bertalot et al.		<0.1%				<0.1%			<0.1%	<0.1%					acidophilic
Stauroneis borrichii (J.B. Petersen) J.W.G. Lund			<0.1%			<0.1%								<0.1%	neutrophilic
Stauroneis muriella J.W.G. Lund						<0.1%									unknown
Stauroneis thermicola (J.B.Petersen) J.W.G.Lund	>5–15%	>15–30%	>15–30%	>30%	>30%	>15–30%	>15–30%	>30%	>30%	>15–30%	>30%	>30%	>30%	>15–30%	neutrophilic
	Non-occurring species	<0.1%	>0.1–5%	>5–15%	>15–30%	>30%									

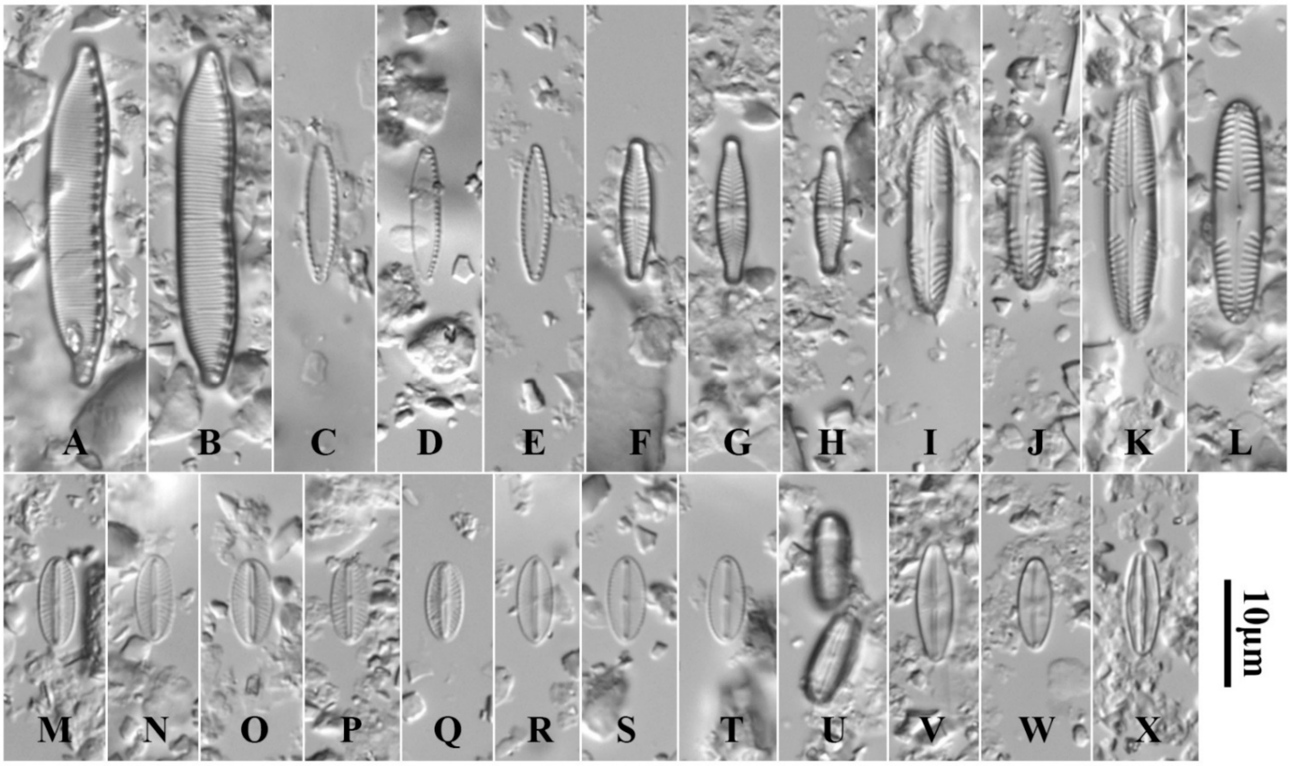

Figure 4. Dominant and some selected diatom taxa observed during the study: (**A**,**B**) Hantzschia amphioxys (Ehrenberg) Grunow; (**C**–**E**) Nitzschia pusilla Grunow; (**F**–**H**) Stauroneis thermicola (J.B. Petersen) J.W.G. Lund; (**I**,**J**) Pinnularia obscura Krasske; (**K**,**L**) Pinnularia schoenfelderi Krammer; (**M**–**Q**) Mayamaea atomus (Kützing) Lange-Bertalot; (**R**–**T**) Mayamaea permitis (Hustedt) K. Bruder and Medlin; (**U**) resting form of unidentified Mayamaea sp.; (**V**,**W**) Sellaphora nana (Hustedt) Lange-Bertalot, Cavacini, Tagliaventi and Alfinito; (**X**) Microcostatus aerophilus Stanek-Tarkowska, Noga, C.E. Wetzel and Ector.

4. Conclusions

The years 2018, 2019, and 2020 were characterized by low total annual precipitation. August, i.e., the month of soil sampling, was dry in both study years, as indicated by the percentage of water content determined in the soil. The doses of ash fertilization did increase the water retention capacity of the podzolic soil.

The pH values in all the combustion ash-fertilized variants did not differ significantly in 2019, whereas the highest pH values in 2020 were found in the variants fertilized with 200 and 300 kg K_2O ha^{-1}. The application of biomass combustion ashes did not increase the reaction in any experimental variant. The pH values in all the variants were higher in the first year of the experiment and decreased in the following year.

Despite the increase in the mean EC values induced by NPK and ash fertilization, compared with the control, the salt concentration in the soil solution was within the range tolerated by all plant species. The application of increasing doses of biomass combustion ash did not increase soil salinity.

Despite the availability of a considerable number of studies on diatoms in terrestrial environments, only a few papers address the ecological preferences of these species. Therefore, there is a need to explore the flora of terrestrial diatoms in natural habitats and areas exposed to constant anthropopressure associated with agricultural activity. Profound knowledge of their preferences and tolerance of environmental parameters may facilitate their future use as potential environmental indicators in different research fields.

Author Contributions: Conceptualization. J.S.-T., M.S.; methodology J.S.-T. and M.S.; investigation. M.R.; writing—original draft preparation. J.S.-T., M.S. and M.R.; formal analysis. J.S.-T. All authors have read and agreed to the published version of the manuscript.

Funding: The research (J.S.-T.; M.S.) on soil material was funded within a subsidy to maintain the research potential of the Department of Soil Science, Environmental Chemistry and Hydrology of the University of Rzeszów for 2018/2019/2020. The research (M.R) was founded by the Polish Ministry of Science and Higher Education under the name of "Regional Excellence Initiative" in 2019–2022 Project No. 026/RID/2018/19 (MR).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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

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

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