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Effects of Strip-Till and Simultaneous Fertilization at Three Soil Depths on Soil Biochemical and Biological Properties

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Abstract: In several studies, the discriminating factor in land use of arable soil is tilling, along with its depth and intensity. Reduced and no-till technologies are held to be beneficial for soil health. Strip-till reduces soil disruption and enables the application of liquid fertilizer directly in rows at different levels. The objective of the research reported here was to evaluate the effects of digestate application on the biochemical and microbiological properties of soil at various soil depths. Three doses of digestate (0, 20, and 40 $m^3 \cdot ha^{-1}$) applied at three different soil depths (0–10, 10–15, and 15–20 cm) were tested in two seasons (2020 and 2021) of semi-operational field trials with maize cultivated according to strip-till practice. In 2020, a lower (20 $m^3 \cdot ha^{-1}$) dose of digestate caused the most significant improvement in β-glucosidase, urease, and basal and L-alanine-induced respiration in topsoil (0–10 cm) and in oxidizable carbon in mid-soil (10–15 cm). In 2021, the most significant positive effect on arylsulfatase, N-acetyl-β-D-glucosaminidase, urease, and all types of respiration were caused by higher (40 $m^3 \cdot ha^{-1}$) digestate dose in mid-soil (10–15 cm). The benefits of the strip-till amended digestate in 2020, as revealed by respiration indicators, strongly decreased with soil depth. Finally, the markedly positive impacts of the digestate applied via the strip-till agromanagement technique were similar for three different depths of soil in 2021, verifying its benefits.

Keywords: digestate; soil respiration; soil enzyme activities; oxidizable carbon

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1. Introduction

Soil physicochemical properties, nutrient contents, and biological properties, which are parameters for assessing soil health, differ in response to changes in land use and the application of various soil amendments [1]. In several studies, land-use types, e.g., farmland, orchard, grassland, and abandoned land, serve as horizontal factors, while soil layers at various depths (e.g., 0–10 cm, 10–30 cm, and 30–60 cm) are used as vertical factors for assessing soil physicochemical and microbial properties [1–4]. The most discriminating factor in arable land use is tilling, the depth of which significantly determines various soil properties, such as soil microbial activity [5–8]; the growth, yield, and water-use efficiency of crops [9]; soil physical properties [8–10]; biochemical properties [11]; soil structural properties used for modeling soil–water behavior [12]; soil faunal groups [6]; and crop yields [5].

Among various tillage practices, reduced tillage (techniques that minimize soil disturbance and leave crop residues or stubble on the soil surface instead of incorporating them into the soil) and no-till technologies are held to have beneficial effects on soil organic carbon content, nutrient transformation [5,11], and soil microbiome enrichment and activity enhancement [5,13,14]. Strip-till is a tillage protection system that minimizes plowing and combines reduced and no-till technologies that retain soil moisture, raise seedbed temperatures, and aerate soil to enable deeper root growth [15]. When mineral fertilizer was added to different soil layers in a micro-field experiment with maize plants, moisture conditions, level of fertility, and root features were found to be application-depth-reliant [16]. Further, biosolid- and tea-waste-derived changes in soil N and P contents were significantly related to the state of soil profiles and erosion ratios [17]. In contrast, strip-till technology enables the application of liquid fertilizer directly to the rows in the soil where the seeds are being planted [18], which reduces the amount of fertilizer needed and improves the proximity of the fertilizer to the roots [19,20]. Thus, among other advantages of strip-till technology is the prevention of soil erosion and the possibility of the simultaneous application of fertilizer at various depths of soil profile.

Digestate is a waste product from biogas production often used in agriculture as a liquid biofertilizer and soil amendment [20] containing significant amounts of minerals, especially nitrogen, phosphorus, and potassium [19]. We hypothesized that digestate application could mediate the following effects:

I. Soil organic matter (SOM) and exogenous organic matter (EOM) degradation rates will be primarily most enhanced in topsoil, although long-term, enhanced activity in topsoil is likely to reduce enzyme activities compared to mid-depth (10–15 cm) soil, due to the consumption of easily utilizable sources and leaching of nutrients from the surface to deeper soil layers.
II. Respiratory carbon mineralization will be highest in topsoil during the whole growing season due to the best aeration and the highest biomass of aerobic microbes.
III. Due to the strip-till application of digestate to the entire soil profile, increased microbial activities will extend to the greatest depth at the highest digestate dose.
IV. Nitrogen mineralization will be lower compared to carbon mineralization at the greatest depth, putatively due to the limited content of organic nitrogen in the digestate and the oxygen-dependency of nitrification processes.

The objective of the research was to compare the effect of digestate, applied simultaneously to all three different soil depths at two different doses (20 and 40 $m^3 \cdot ha^{-1}$), on biochemical and microbiological properties and their rates of change as functions of depth.

2. Materials and Methods

2.1. Site Description and Experimental Design

In the years 2020 and 2021, the experiment was carried out in fields near the village of Senice na Hané (49°37′32.5″ N 17°05′14.2″ E). The site is located in a fertile area of Hornomoravský úval (Olomouc District, Czech Republic), at an altitude of approximately 300 m a.s.l. The area belongs to the warm T2 climatic region, which is characterized by long, warm, dry summers and short, mild winters. The average air temperature is −2 to −3 °C in January and 18 to 19 °C in July. Rainfall in the growing season is between 350–400 mm and 200–300 mm in winter. The soil type is chernozem, and the soil texture (according to the USDA soil texture triangle) is silt loam. The soil properties are displayed in Table 1.

Table 1. Chernozem soil properties at the experimental site.

Year		pH (-)	Cox (%)	Ntot (%)
2020	Mean	6.87	1.09	0.16
	SD	0.09	0.02	0.00
2021	Mean	7.14	1.14	0.14
	SD	0.02	0.03	0.00

At the beginning of September 2019, after the cereal harvest, a mixture of stubble intercrops was sown at a rate of 10 $kg \cdot ha^{-1}$ of white mustard (*Sinapis alba*) and 5 $kg \cdot ha^{-1}$ of lacy phacelia (*Phacelia tanacetifolia*), according to the usual agrotechnical methods. The intercrops were left in the field, where they froze over the winter, and their remains were mulched in the spring. A full-scale fertilization with NPK fertilizers (160 kg N·ha^{-1}) was carried out in spring 2020.

In mid-April 2020, semi-operational plots of approximately 0.50 ha with different treatments were established by the simultaneous application of digestate at 3 depths of 10, 15, and 20 cm, at doses of 0, 20, and 40 m^3·ha^{-1}. The application of digestate with nitrification inhibitor VIZURA (BASF Ltd., division, Prague, Czech Republic) was carried out using a VT4556 machine (Vredo Dodewaard B.V, Dodewaard, The Netherlands) equipped with a MUCK TILLER applicator (P & L, spol. s r.o., Biskupice, Czech Republic) for strip-tillage and digestate application. One week after the digestate application, the maize (*Zea mays*) hybrid Banshee (FAO 300) was sown. The maize was harvested 5 months after sowing. Soil sampling was carried out one week after harvest.

In early September 2020, intercrops were sown again and left to freeze, as in the previous year. In 2021, the same operations were followed for the experimental plot as in the first year of the experiment.

The properties of the applied digestate are given in Table 2.

Table 2. The properties of the applied digestate.

Year		DM (%)	N/NH$_4$ in FM (g·kg^{-1})	N/NO$_3$ in FM (g·kg^{-1})	N$_{min}$ in FM (g·kg^{-1})	P in FM (g·kg^{-1})	K in FM (g·kg^{-1})
2020	Mean	3.943	1.514	0.491	2.005	0.159	3.738
	SD	0.080	0.057	0.017	0.074	0.010	0.217
2021	Mean	7.100	3.390	0.305	3.695	0.408	2.953
	SD	0.377	0.036	0.005	0.033	0.024	0.151
2021 vs. 2020	Relative values	1.8	2.2	0.6	1.8	2.6	0.8

DM = dry matter of digestate; FM = fresh matter of digestate; N/NH$_4$ = ammonium nitrogen; N/NO$_3$ = nitrate nitrogen; N$_{min}$ = inorganic nitrogen; P, K = phosphorus, potassium; mean (n = 3) and standard deviation (SD) values and relative values (comparison between both digestates).

2.2. Soil Sampling and Measurements

The experimental plots were transversely divided into three parts. A soil sample was taken at depths of 0–10 cm, 10–15 cm, and 15–20 cm from each part. Thus, three individual soil samples merged from 8 sampling points were obtained for each treatment and for each depth. In the laboratory, samples were homogenized, sieved (≤2 mm), and air-dried for the measurement of oxidizable carbon (Cox), according to [21]. Freeze-dried samples were used for the following enzyme activity assays [22]: β-glucosidase (GLU), N-acetyl-β-D-glucosaminidase (NAG), arylsulfatase (ARS), and urease (Ure). Samples cooled to 4 °C were used for basal respiration (BR) and substrate-induced respiration determinations, using a MicroResp device, according to [23]. Substrate-induced respiration was measured after adding specific energy sources to the substrate: D-glucose (Glc-SIR), D-mannose (Man-SIR), and L-alanine (Ala-SIR).

2.3. Statistical Analysis

Data processing and statistical analyses were performed using the freely available software R, version 3.6.1 [24]. For characterization of the relationships between the treatments and selected soil properties, one-way and two-way analyses of variance (ANOVAs) were used, both of type I (sequential) sum of squares at a 5% significance level. For the detection of statistically significant differences among factor level means, Tukey's HSD (honestly significant difference) test was used, and "treatment contrast" was performed to calculate factor-level means for each treatment. The results were graphically presented using a simple barplot with standard deviations (SDs) together with statistically significant differences among variants. To check the statistical models, the Kolmogorov–Smirnov test (for normality-distribution checking) and Bartlett's test (for checking the heteroscedasticity of residuals) were used, also at a significance level of 0.05. In addition, different diagnostic (e.g., Q–Q) plots were also used for the purpose. For advanced statistical modelling of the dependence relationships between soil properties and treatments, principal component analysis (PCA) was also applied. Eigenvalues were used to measure the amount of variation retained by each principal component. These results were graphically presented with the help of Rohlf biplots for standardized PCAs. Pearson correlation analysis was performed to measure the linear dependences between soil properties. Pearson correlation coefficients were interpreted as follows: 0.0 < r < 0.3 (negligible correlation), 0.3 < r < 0.5 (low correlation), 0.5 < r < 0.7 (moderate correlation), 0.7 < r < 0.9 (high correlation), and 0.9 < r < 1.0 (very high correlation).

3. Results

3.1. Soil Oxidizable Carbon and Enzyme Activities

Control unamended soil (0 $m^3 \cdot ha^{-1}$) showed significantly decreased oxidizable carbon (Cox) at depths of 10–15 cm (compared to a digestate dose of 20 $m^3 \cdot ha^{-1}$) in 2020. Seasons for 2021 revealed Cox decrease (average, insignificant values) in the control (0 $m^3 \cdot ha^{-1}$) compared to the digestate treatment of 20 $m^3 \cdot ha^{-1}$ at depths of 10–15 and 15–20 cm (Figure 1a). A trend of decreasing Cox at depths of 0–10 cm and 10–15 cm was revealed for a digestate dose of 0 $m^3 \cdot ha^{-1}$ and for a digestate dose of 20 $m^3 \cdot ha^{-1}$ between 10–15 cm and 15–20 cm in both the 2020 and 2021 seasons. Cox decreased significantly, also, with a digestate dose of 40 $m^3 \cdot ha^{-1}$ in 2021 at soil depths of 10–15 cm and 15–20 cm. We identified a generally positive effect of digestate amendment dose on Cox content in deeper soil layers, mainly mid-depth (10–15 cm) in both years. On the contrary, no positive effect of digestate dose amended to the topsoil was observed. Moreover, digestate amendment to the topsoil in 2021 decreased Cox in the topsoil (Figure 1a).

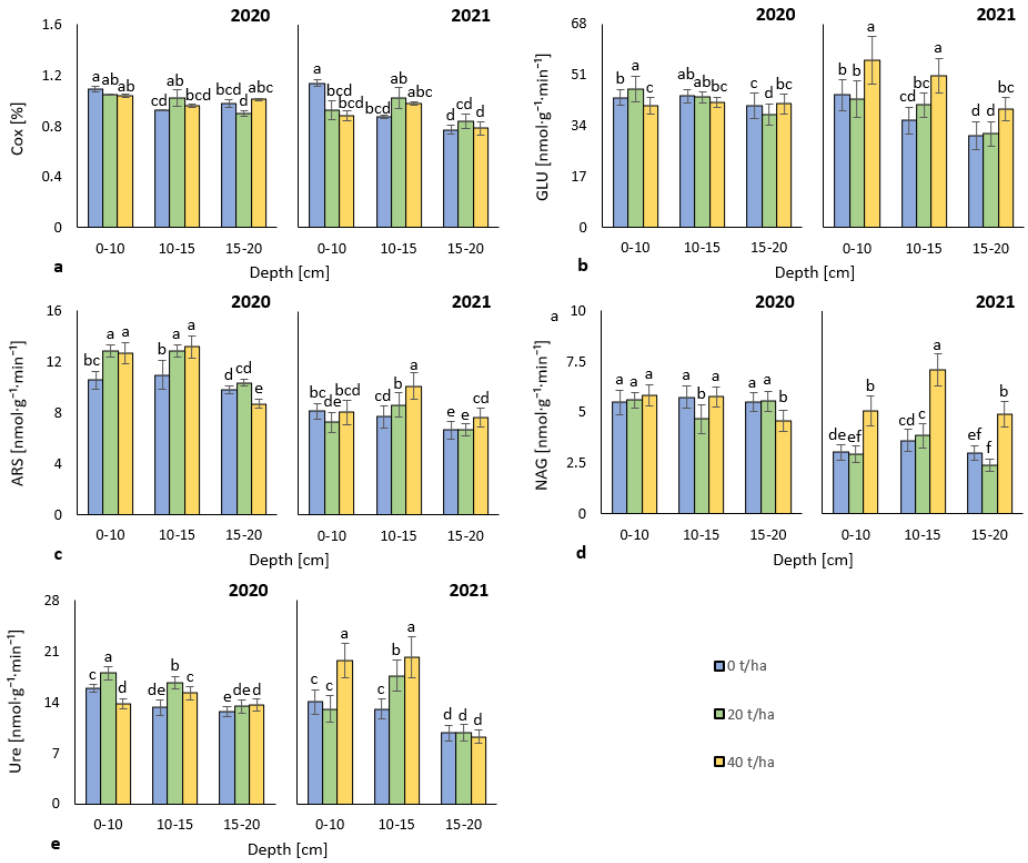

Figure 1. (**a**) Oxidizable carbon and activity of (**b**) β-glucosidase, (**c**) arylsulfatase, (**d**) N-acetyl-β-D-glucosaminidase, (**e**) and urease at the various depths of soil amended with different doses of digestate (0, 20, and 40 $m^3 \cdot ha^{-1}$) in the years 2020 and 2021. Different letters indicate differences at a level of significance of $p \leq 0.05$.

β-glucosidase (GLU) activity, an indicator of plant waste carbon mineralization, declined at a depth of 15–20 cm as compared to topsoil at all doses (except 40 $m^3 \cdot ha^{-1}$ in 2020) in both the 2020 and 2021 seasons, closely similar to trends in values for Cox in 2020 (Figure 1b). GLU at a depth of 15–20 cm in unamended soil was negatively affected by year, as evident from higher values in 2020 compared to 2021. Comparable GLU values among all digestate doses at the mid-depth of 10–15 cm were detected in 2020. On the contrary, the positive impact of a high digestate dose (40 $m^3 \cdot ha^{-1}$) on GLU was statistically significant at all three depths of soil in 2021.

Arylsulfatase (ARS), which is involved in the mineralization of organosulfates, significantly decreased values in the deepest layer (15–20 cm) of chernozem as compared to topsoil and depths of 10–15 cm (for all three tested doses) in 2020 (Figure 1c). Mid-depth soil (10–15 cm) doses of 20 and 40 $m^3 \cdot ha^{-1}$ showed significant increases in ARS in comparison to topsoil and 15–20 cm depths in 2021 (Figure 1c). Digestate doses of 20 and 40 $m^3 \cdot ha^{-1}$ caused significantly higher ARS values compared to 0 $m^3 \cdot ha^{-1}$ (i.e., positive impacts of doses) in topsoil and depths of 10–15 cm in 2020, but only at depths of 10–15 cm in 2021. A negative effect of digestate application year was evident with respect to ARS, which was deducible from higher values (except for 40 $m^3 \cdot ha^{-1}$ in 10–15 cm) for 2020 as compared to 2021.

N-acetyl-β-D-glucosaminidase (NAG) is an indicator of nitrogen and carbon mineralization related to fungal biomass turnover, which values were also significantly affected by soil depth: increased NAG was found at depths of 10–15 cm with doses of 20 and 40 $m^3 \cdot ha^{-1}$ (compared to topsoil and depths of 15–20 cm) and with 0 $m^3 \cdot ha^{-1}$ treatment (compared to treatment at depths of 15–20 cm) in 2021 (Figure 1d). In contrast, NAG at 20 $m^3 \cdot ha^{-1}$ at 10–15 cm was significantly decreased (compared to 0 and 40 $m^3 \cdot ha^{-1}$) in 2020. A significant enhancement of NAG was revealed at 40 $m^3 \cdot ha^{-1}$ for all three depths in comparison to 0 and 20 $m^3 \cdot ha^{-1}$ in 2021 (i.e., a positive impact of digestate dose). A negative effect of digestate application year on NAG was evident, which was deducible from higher values (at 0 and 20 $m^3 \cdot ha^{-1}$ doses) in 2020 as compared to 2021.

Urease (Ure) is a ubiquitous enzyme used to monitor the early phase of nitrogen mineralization and deamination of organic nitrous compounds. Ure activity was significantly decreased (compared to topsoil) in the deepest layer of 15–20 cm at doses of 0 and 20 $m^3 \cdot ha^{-1}$ in 2020 and at all doses in 2021 (Figure 1f). A negative effect of application year on Ure activity in the deepest soil (15–20 cm) was also indicated. In 2020, a dose of 20 $m^3 \cdot ha^{-1}$ increased Ure activity in topsoil and mid-depth soil (10–15 cm) compared to doses of 0 and 40 $m^3 \cdot ha^{-1}$. Significantly increased Ure in mid-depth soil (10–15 cm) with a dose of 20 $m^3 \cdot ha^{-1}$ (compared to treatments at depths of 0–10 cm and 15–20 cm) was revealed in 2021, and a dose of 40 $m^3 \cdot ha^{-1}$ enhanced Ure (i.e., a positive effect of digestate dose) in topsoil and in soil at a depth of 10–15 cm (compared to 15–20 cm) in 2021 (Figure 1d).

3.2. Soil Respiration

Soil basal respiration (BR) indicates aerobic catabolic carbon-mineralizing activity of soil microbiomes. It was significantly decreased in the deepest soil (15–20 cm) under digestate doses of 20 and 40 $m^3 \cdot ha^{-1}$ (2020) compared to topsoil and compared to topsoil and soil depths of 10–15 cm at 20 $m^3 \cdot ha^{-1}$ (2021) (Figure 2a). In 2020, comparison of the topsoil and mid-depth values at doses of 20 and 40 $m^3 \cdot ha^{-1}$ showed negative effects of the applied digestate doses on BR. A dose of 40 $m^3 \cdot ha^{-1}$ improved BR in topsoil and at soil depths of 10–15 cm (compared to 0 and 20 $m^3 \cdot ha^{-1}$) in 2021. A positive effect of application year at a dose of 40 $m^3 \cdot ha^{-1}$ was evident from a comparison of values for 2020 and 2021 in topsoil and at soil depths of 10–15 cm.

D-glucose-induced respiration (Glc-SIR) is related to the total respiration capacity of the soil microbiome and to the whole biomass of aerobic carbon-mineralizing microorganisms in soil. The effect of depth on Glc-SIR was positive in unamended soil and negative at a dose of 20 $m^3 \cdot ha^{-1}$ and marked at 40 $m^3 \cdot ha^{-1}$ from a comparison of values for 2020

(Figure 2b). Significant positive effects of the applied doses of digestate on Glc-SIR were exerted in topsoil in 2020. Comparison of Glc-SIR values at 0 and at 40 $m^3 \cdot ha^{-1}$ at all three depths showed positive effects of the applied digestate doses, even in 2021. Glc-SIR was significantly higher at soil depths of 15–20 cm compared to topsoil and at depths of 15–20 cm at 0 and 20 $m^3 \cdot ha^{-1}$ in 2021, which indicated positive effects of the application doses. Higher values for the deepest soil (15–20 cm) in 2021 compared to 2020 also showed a positive effect of application year on Glc-SIR values (Figure 2b).

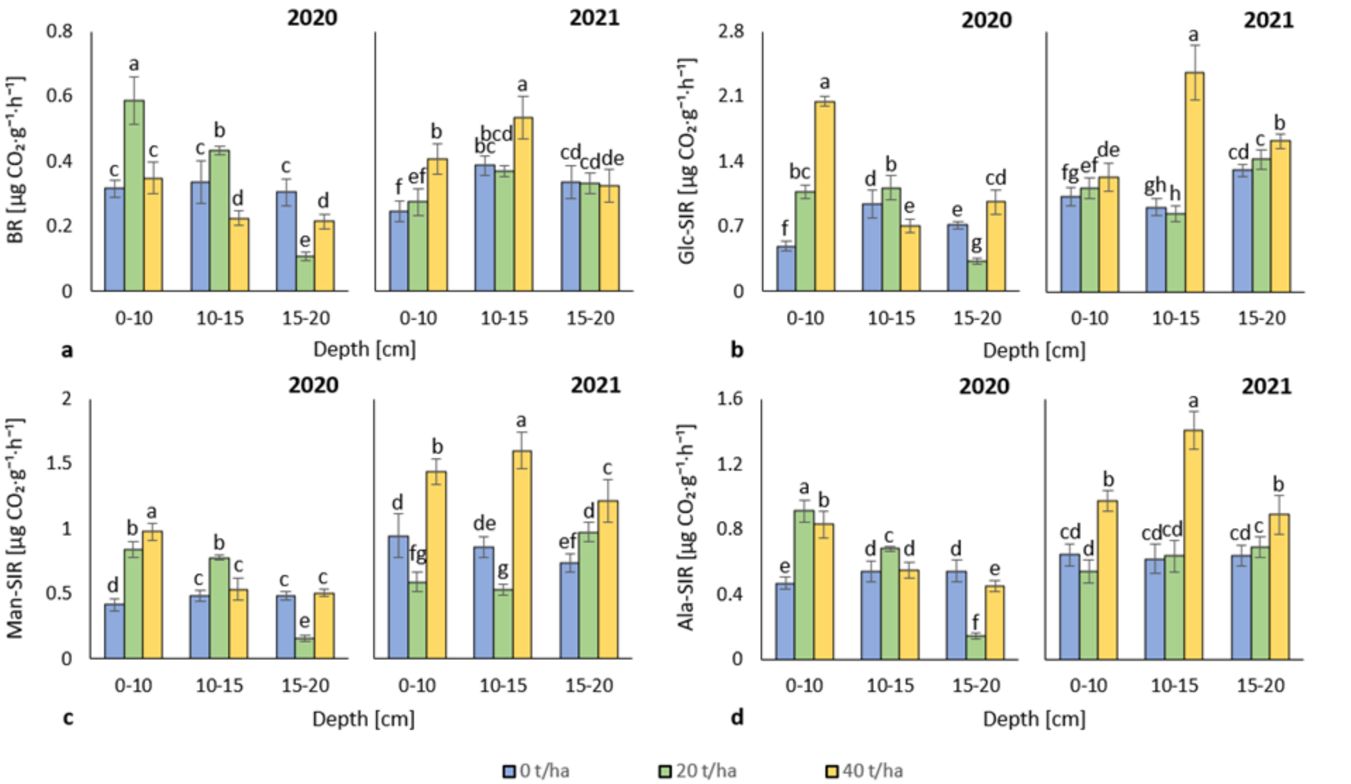

Figure 2. (**a**) Basal respiration and respiration induced by (**b**) D-glucose, (**c**) D-mannose, and (**d**) L-alanin at various depths of soil amended with different doses of digestate (0, 20, and 40 $m^3 \cdot ha^{-1}$) in the years 2020 and 2021. Different letters indicate differences at a level of significance of $p \leq 0.05$.

D-mannose (Man-SIR)- and L-alanine (Ala-SIR)-induced respiration showed similar results, albeit they indicated either carbon- or carbon + nitrogen-related aerobic mineralization processes in the soil, respectively. Markedly increased values for both Man-SIR and Ala-SIR were detected at 0 and 40 $m^3 \cdot ha^{-1}$ at all depths in 2021 compared to values for 2020, which proved a positive effect of application year on these SIRs (Figure 2c,d). Both Man-SIR and Ala-SIR values at 20 and 40 $m^3 \cdot ha^{-1}$ showed a negative effect of depth (mainly if topsoil and 15–20 cm soil depths were compared) in 2020 (Figure 2c,d). Under digestate amendment of 20 $m^3 \cdot ha^{-1}$, a positive effect of application depth was shown also for Man-SIR in 2021. Man-SIR and Ala-SIR were significantly highest at all depths at a dose of 40 $m^3 \cdot ha^{-1}$ compared to other doses for 2021 (Figure 2c), which proved a positive effect of application dose. Mid-depth (10–15 cm) soil showed the highest Man-SIR and Ala-SIR values compared to topsoil and deep soil at 40 $m^3 \cdot ha^{-1}$.

Figures 3 and 4 display the results of Pearson's correlation analyses, which evaluated relations between the determined microbial activities and which are discussed (for each soil parameter) in the Discussion section.

On the basis of the results of the PCAs (Figure 5), we can conclude that, from the values for the 2020 season, approximately 77% of the variation is explained by the first two eigenvalues together: the first eigenvalue explains 61% and the second explains 16%. Along the first dimension, the following microbial activities make large contributions:

Ala_SIR (17%), Man_SIR (16%), BR (15%), and Cox (14%). Along the second dimension, the following microbial activities make large contributions: NAG (42%), Glc_SIR (14%), and GLU (14%). The total contributions of microbial activities regarding PC1 and PC2 together are as follows (in descending order): Ala_SIR, Man_SIR, Cox, and BR. Other microbial activities do not make large contributions in accounting for the variability with respect to the first two principal components.

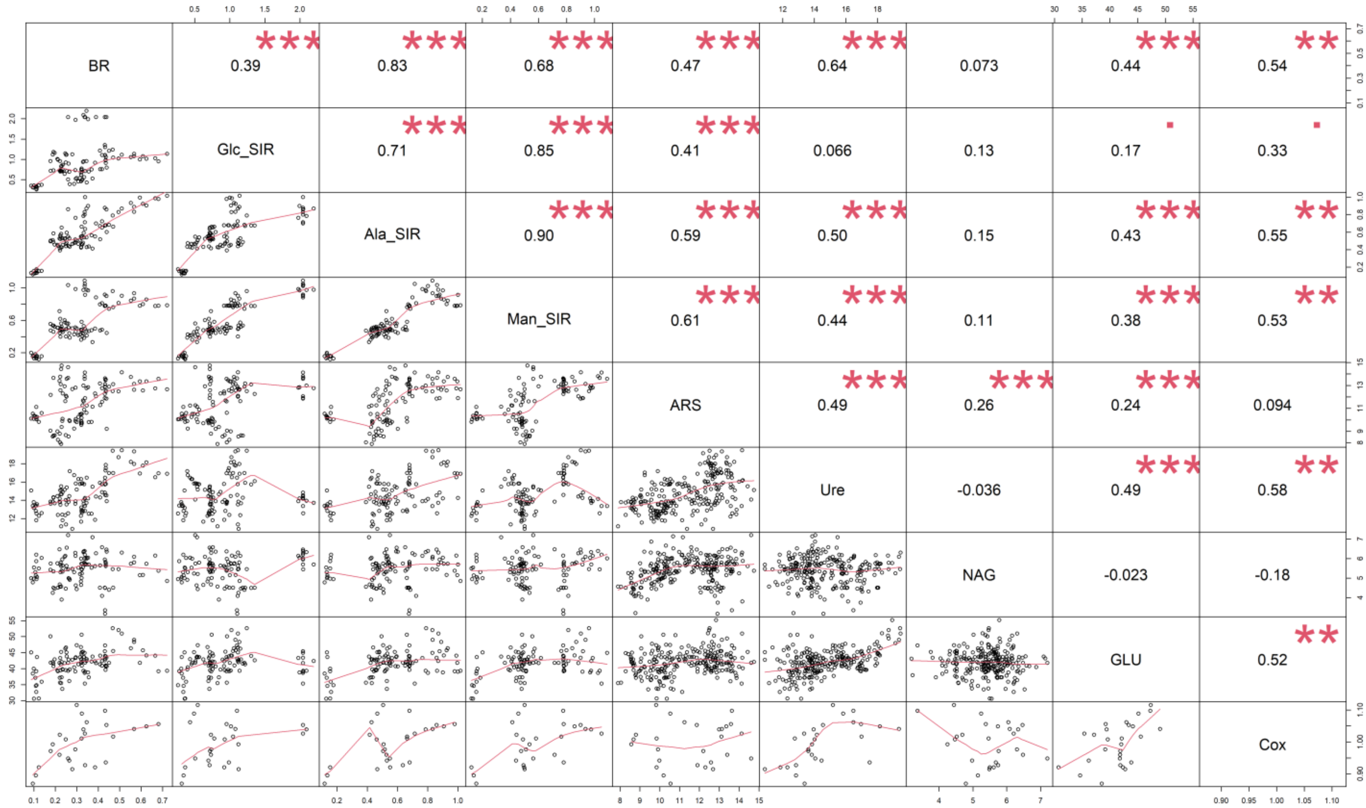

Figure 3. Pearson's correlation analyses of values for the 2020 season. The stars indicate differences between the variables at the following levels of statistical significance: ** $p \leq 0.01$, *** $p \leq 0.001$.

From the results of the PCAs (Figure 6), we can conclude that, given the values from the 2021 season, approximately 82% of the variation is explained by the first two eigenvalues together: the first eigenvalue explains 59% and the second explains 23%. Along the first dimension, the following microbial activities make large contributions: Ala_SIR (17%), NAG (16%), Man_SIR (13%), BR (12%), and ARS (11%). Along the second dimension, the following microbial activities make large contributions: Cox (39%), Glc_SIR (17%), Ure (13%), and GLU (12%). The total contributions of microbial activities regarding PC1 and PC2 together are as follows (in descending order): Ala_SIR, Cox, NAG, Glc_SIR, and Ure. Other microbial activities do not make large contributions in accounting for the variability with respect to the first two principal components.

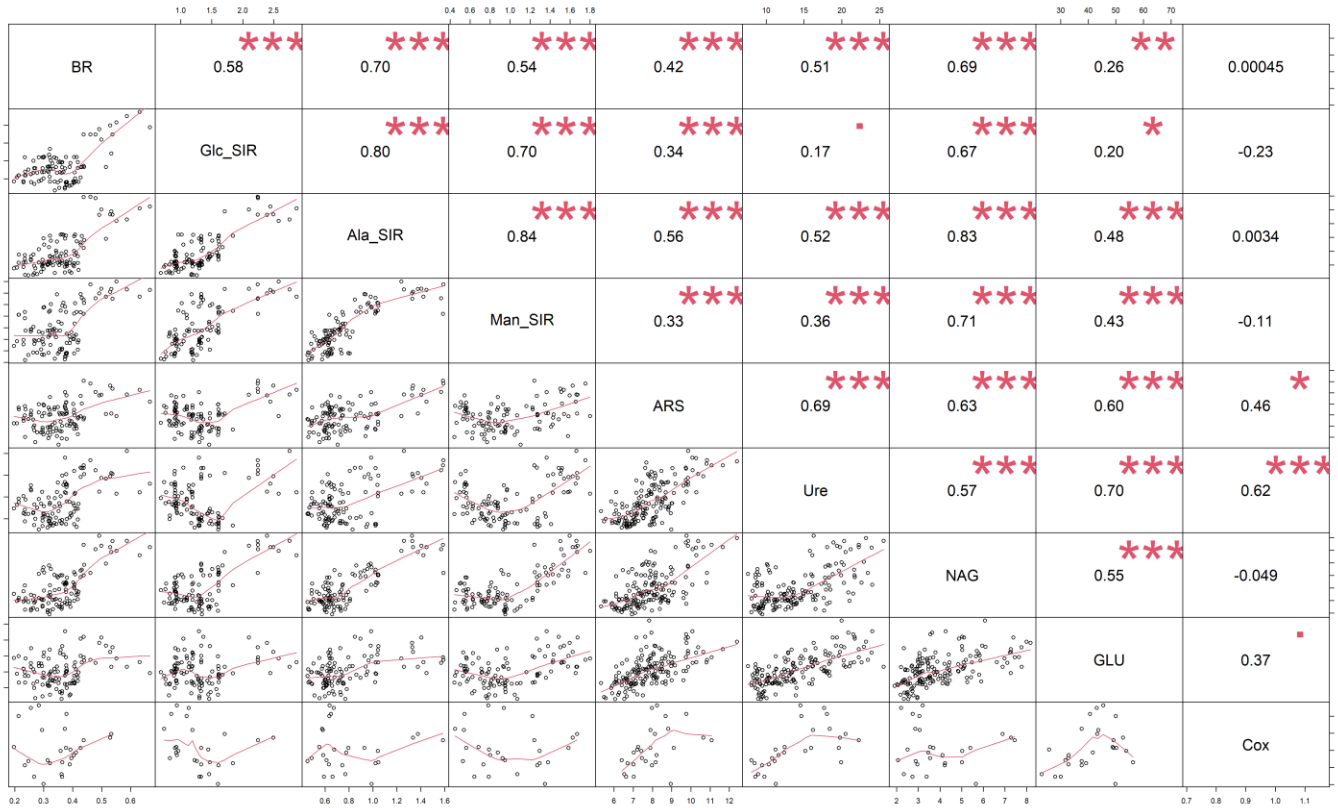

Figure 4. Pearson's correlation analyses of values for the 2021 season. The stars indicate differences between the variables at the following levels of statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

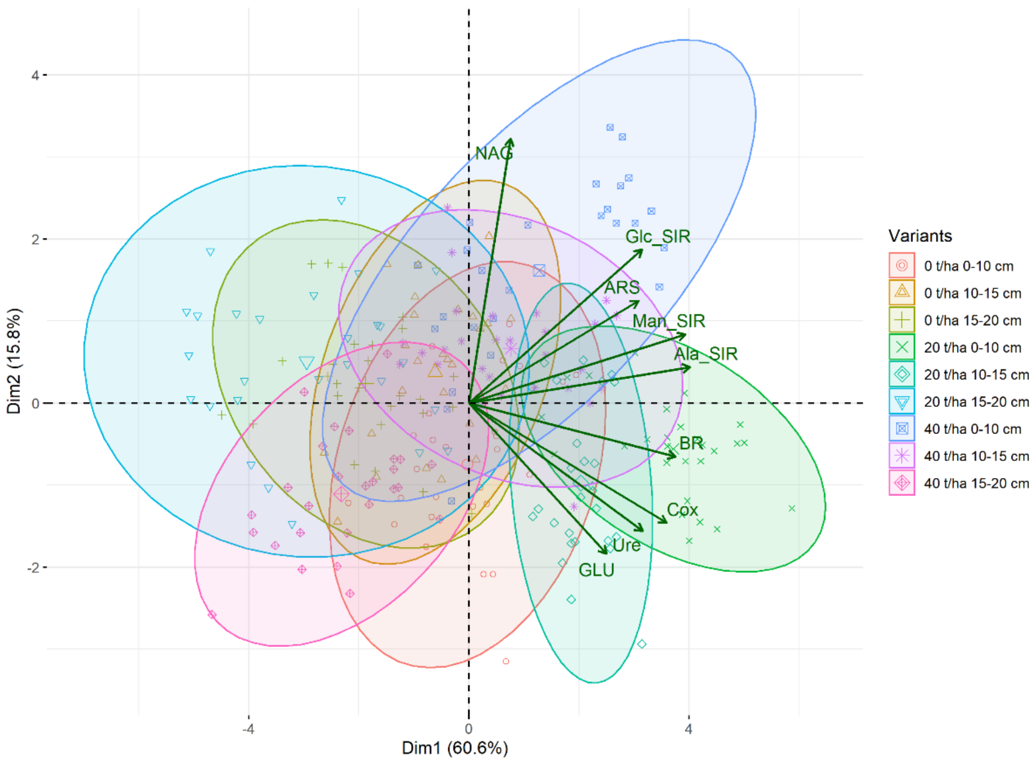

Figure 5. Rohlf's principal component analysis biplot of values for the 2020 season.

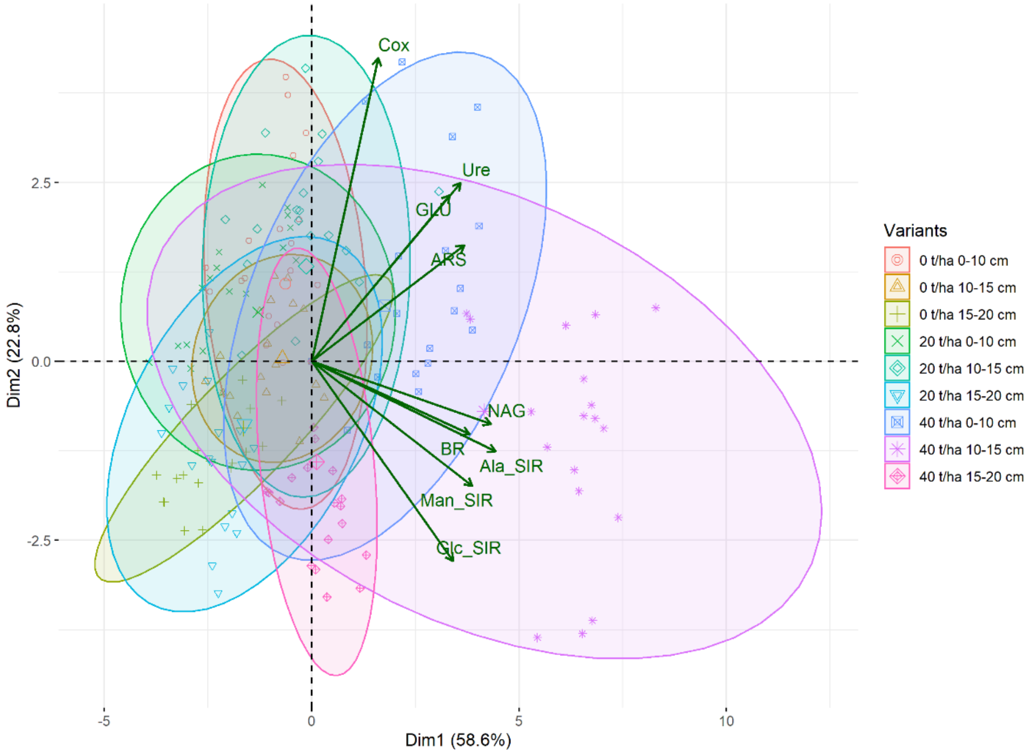

Figure 6. Rohlf's principal component analysis biplot of values for the 2021 season.

4. Discussion

4.1. Soil Oxidizable Carbon and Enzyme Activities

Soil oxidizable carbon (Cox), which was reported to be related to microbially available (and utilizable) carbon [25], was found to be significantly decreased in deeper layers (10–15 and 15–20 cm) compared to topsoil (0–10 cm) in both unamended (0 $m^3 \cdot ha^{-1}$) treatments for 2020 and 2021. There was a significant decline in Cox at soil depths of 10–15 and 15–20 cm under the digestate treatments of 20 $m^3 \cdot ha^{-1}$ (in 2020 and 2021) and 40 $m^3 \cdot ha^{-1}$ (in 2021). The depth-dependent differences in Cox were expectable under the strip-till system, as Diederich et al. (2019) previously reported similar, 50% greater Cox values in topsoil (0–15 cm) compared to 15–30 cm depths [26]. These differences may be due majorly to the application of digestate having been carried out at a depth of 10–15 cm, such a procedure leading to the accumulation of available organic carbon in this soil layer. Furthermore, Ma et al. (2020) observed an indirect relation between depth and carbon available for mineralization [27].

Slepetiene et al. (2020) observed that the application of anaerobic digestion (AD) product to soil (0–40 cm depth) increased organic carbon (mobile humic acids) mainly in topsoil. However, in our work, we did not reveal any positive effect of digestate amendment on Cox values in topsoil, regardless of digestate composition. The lack of effect on Cox values was possibly due to a high percentage of original SOM in the upper soil layer. Further, we presumed significantly enhanced mineralization of digestate-derived labile carbon fractions (carried out by aerobic microorganisms, documented by respiration results; see Section 3.2) in upper layers compared to deeper soil layers. This assumption, proven by comparison of (Cox and respiration) results for soil depths of 10–15 cm and 15–20 cm at a 20 $m^3 \cdot ha^{-1}$ digestate dose in the 2020 season, was in line with the reported higher mineralization rate of external carbon in surface soil (compared to when it was incorporated into the soil) after treatment with sewage sludge digestate [28]. Such an assumption of mutually related carbon (aerobic) mineralization rate and availability of labile carbon substrate (Cox) was corroborated by significant ($p \leq 0.01$) positive correlations of Cox with basal respiration (BR) and substrate-induced respiration (with D-mannose and L-alanine) in 2020 (r = 0.54, 0.55, 0.53; Figure 3).

Although digestate application did not affect Cox content in topsoil, mid-soil at a depth of 10–15 cm was positively affected in both 2020 and 2021 by digestate at a dose of 20 $m^3 \cdot ha^{-1}$ compared to the control treatment (0 $m^3 \cdot ha^{-1}$). We concurrently detected significant increments in respiration values in the 10–15 cm layer (compared to topsoil) at all digestate doses (BR) and at 40 $m^3 \cdot ha^{-1}$ (all SIRs) in 2021. Therefore, we ascribe an apparently contributing effect to strip-till incorporation of digestate into the soil profile (the 10–15 cm layer) on Cox content in soil and its longer-lasting preservation for extended enhancement of carbon mineralization and overall organic matter transformation. We corroborated this with significant Cox synergy (on a PCA biplot) and correlation ($p \leq 0.01$) with GLU (r = 0.52) in 2020 (Figures 3 and 5). Contrary to Cox, β-glucosidase (GLU) activity in topsoil responded to the digestate amendment positively in both 2020 (at 20 $m^3 \cdot ha^{-1}$) and 2021 (at 40 $m^3 \cdot ha^{-1}$), which indicated stimulation due to access to more recalcitrant organic carbon (AD-transformed lignocellulose material). The stimulatory effect of digestate on GLU activity is known [29], and we revealed a positive effect of digestate (in 2021) on GLU values in 2021 at all depths with a dose of 40 $m^3 \cdot ha^{-1}$ (Figure 1b) in comparison to unamended control soil. Plaza et al. (2004) reported, also, that high access to EOM from pig slurry significantly enhanced soil GLU and other enzyme activities, regardless of the metabolic quotient and total organic carbon [30]. We inferred from this that strip-till application enabled the introduction of digestate-derived EOM in high amounts into the entire treated soil profile, which preserved cellulolytic activity until the end of the vegetation period. We did not verify our hypothesis I, but we corroborated hypothesis III.

As well as GLU, other enzymes (arylsulfatase, N-acetyl-β-D-glucosaminidase, and urease) showed significant ($p \leq 0.001$) positive correlations (ARS r = 0.60, NAG r = 0.55, Ure r = 0.70) and synergy (GLU, ARS, and Ure on the PCA biplot) for 2021 (Figures 4 and 6). These results are in line with those of Meyer et al. (2015), who referred to increased Ure and GLU activities in the soil under organic treatments (compost and straw mulch) compared to conventional treatments (synthetic fertilizer and herbicide) at all depth intervals [31].

The results for ARS showed that mineralization of sulfur was negatively dependent on soil depth in the unamended treatment (Figure 1c). It has already been reported that ARS activity decreased markedly with depth in six soil profiles [32], and this decrease was associated with a decrease in organic carbon content [33]. ARS values were those most enhanced by digestate addition in the topsoil and mid-depth soil (10–15 cm) in 2020 at both digestate doses. In 2021, ARS enhancement was found in mid-depth (both digestate doses) and deep soil (40 $m^3 \cdot ha^{-1}$). The findings for 2020 were in line with those of a previous study [34], where ARS activity showed a strong relationship with microbial biomass carbon and organic carbon under pig slurry amendment in topsoil (0–15 cm). The positive effect of strip-till application of digestate on the stimulation of nutrient-transformation activities in deeper soil was proven. The results for the 2021 season were in line with reported increments in ARS values with greater soil depth [34] but in contrast to the results of another study which did not find any effect of liquid hog manure application on enzyme activities at a depth of 15–30 cm [35].

Determination of N-acetyl-β-glucosaminidase (NAG) showed a minimal effect of soil depth or digestate dose on fungal turnover in 2020. On the contrary, digestate incorporated into soil at a high dose (40 $m^3 \cdot ha^{-1}$) significantly improved NAG activity at all depths (Figure 1d) in 2021, probably due to the higher accessibility of nutrients. In the past, digestate was found to enhance NAG activity in soil [36]. We inferred from these results that strip-till treatment of less rich soil impacted fungal biomass within the full profile positively, without any negative effect of soil depth. Our observation was in contrast with the results of [14], whose authors applied conventional tillage instead of strip-till methods and observed a negative dependence of fungal biomass on soil depth. We assumed that the digestate-derived nitrogen supplement supported fungal growth more efficiently in mid-soil compared to topsoil (2021) due to the reduced volume of ammonia volatilization (causing nitrogen losses). Furthermore, oxygen saturation in different soil layers may affect putative NAG-related carbon and nitrogen aerobic mineralization because these processes are coupled and succeed chitin degradation by NAG. Pearson's correlations for 2021 also revealed significant ($p \leq 0.001$) positive correlations of NAG with BR, Glc-SIR, Man-SIR, and Ala-SIR (r-values were 0.69, 0.67, 0.71, and 0.83) (Figure 4). Thus, the mutual dependence of NAG activity, respiration rate, and oxygen saturation in soil was expected.

Urease (Ure) is an enzyme which generates ammonium and is involved in organoamine mineralization. Ure declined with depth (to the significantly lowest values at depths of 15–20 cm) in both unamended soil types and in 2020 with a dose of 20 $m^3 \cdot ha^{-1}$ (Figure 1e). The reason was a limited amount of SOM, putatively decreased in the deepest soil layer, similarly to Li et al.'s (2015) reference to SOM decline with depth, which was coupled with lower contents of microbial necromass [37]. However, the digestate treatment in 2020 increased Ure values at a dose of 20 $m^3 \cdot ha^{-1}$ in topsoil and soil depths of 10–15 cm, whereas nutrient-richer digestate in 2021 increased Ure most in mid-soil (20 and 40 $m^3 \cdot ha^{-1}$) and in topsoil only at higher application dose (40 $m^3 \cdot ha^{-1}$). Therefore, we again determined that the demand for digestate-derived ammonium nitrogen in mid-soil compared to topsoil was higher in 2021, probably due to the lower accessibility of nitrate nitrogen. Although SOM and EOM transformation were enhanced, nitrogen immobilization could occur in the treated soil due to the high SOM–mineral N ratio (see Table 2). Priming adverse effect of digestate amendment on higher ammonium losses via volatilization, the importance of which was reported in [38], could also play a minor role. Nevertheless, the increased access of digestate to the soil microbiome induced Ure activity, as was found for treated soil in the phase of crop maturity [39]. The results of the enzyme determination enabled us to prove hypothesis III and, at least for ARS, NAG, and Ure, hypothesis IV, too.

4.2. Soil Respiration

Soil basal respiration (BR) monitored carbon mineralization at various soil depths and showed, surprisingly, no difference within the full profile for 2020 and decline in the topsoil compared to deeper layers for the control treatment in 2021. In agreement with the results for other microbial activities, respiration in the 2021 season was highest in mid-soil at 40 $m^3 \cdot ha^{-1}$, and the same dose significantly increased, also, in the topsoil (compared to depths of 15–20 cm) (Figure 2a). This result was in line with previously reported positive effects of digestate addition on BR [40,41]. The soil in 2020 showed higher BR in topsoil and mid-soil at 20 $m^3 \cdot ha^{-1}$, but with a clear negative dependence of values on increasing depth and lower BR at soil depths of 10–15 cm and 15–20 cm (compared to topsoil) at a dose of 40 $m^3 \cdot ha^{-1}$. These results proved our hypothesis II and indicated that digestate applied to all soil layers did not induce the equal stimulation of aerobic carbon mineralization, presumably on account of lower deep-soil aeration and less carbon accessibility, due to the lower dry mass of the digestate, acting as limiting factors on BR in 2020. Similar findings have been reported. Fang and Moncrieff (2005) found that soil carbon pools and microbial respiratory activity, as well as CO_2:TOC (total organic carbon) ratios, declined rapidly with soil depth [42]. Spohn and Chodak (2015) reported that a change in metabolic quotient with increasing depth was not controlled solely by soil carbon concentration [43]. Nevertheless, these features were not observed in 2021.

Due to putatively more significant stimulation of the microbiome by high doses of richer digestate (higher nutrient and DM contents) in 2021, derived EOM promoted microbial growth and an increase in total respiratory potential (abundance of aerobes), as indicated by Glc-SIR. This higher biomass of respirating microbes could overcome oxygen limitation. We can infer this from Glc-SIR values for 2021, which were significantly higher at all depths with 40 $m^3 \cdot ha^{-1}$ treatments (compared to unamended soil at depths of 10–15 cm and 15–20 cm, also compared to doses of 20 $m^3 \cdot ha^{-1}$) (Figure 2b). It was again in line with a reported higher positive effect of digestate on substrate-induced respiration in soil parallelly treated with digestate and mineral fertilizer [40]. Absolutely highest Glc-SIR values were found in mid-soil (10–15 cm), which is explainable by the presumed significantly enhanced mineralization of the digestate-derived labile carbon fraction in topsoil compared to deeper soil layers, which preserved more oxidizable carbon for increased durations of digestate-coupled stimulation of aerobic respiration microbes.

In contrast to 2021, Glc-SIR in 2020 was significantly most increased in topsoil by both digestate doses but also greater soil depth. This fact emphasized the positive effect of digestate amendment in topsoil but indicated the decreased effect of amendment at deeper layers. Apparently, the digestate stimulation of respiration capacity in deeper layers was again limited by oxygen saturation. Nevertheless, significant increments in Glc-SIR in the deepest soil (15–20 cm) in 2020 and all SIRS increments (detected at 15–20 cm depths) at the high dose 40 $m^3 \cdot ha^{-1}$ (compared to 0 and 20 $m^3 \cdot ha^{-1}$) in 2021 indicated a positive contribution of strip-till digestate application to the enrichment of all soil layers with nutrients and for stimulation of their transformation. The mutual relations between all determined types of respiration and microbial activities were evidenced by significant ($p \leq 0.001$) positive correlations of BR with Glc-SIR (r = 0.39, 2020; r = 0.58, 2021), Man-SIR (r = 0.68, 2020; r = 0.54, 2021), Ala-SIR (r = 0.83, 2020; r = 0.70, 2021), and with Ure (r = 0.64, 2020; r = 0.51, 2021) and ARS (r = 0.47, 2020; r = 0.42, 2021).

Therefore, similar but even stronger (than Glc-SIR) positive effects of rich digestate amendment on sugar- and amino-acid induced respiration (Man-SIR and Ala-SIR) in 2021 were observed. Man-SIR and Ala-SIR caused significant enhancement of these SIRs at all depths with treatments of 40 $m^3 \cdot ha^{-1}$ and at 15–20 cm (for Man-SIR) (Figure 2c,d). In 2021, the highest values of Man-SIR and Ala-SIR were found in mid-soil, from which we assumed (as with the Ure determination results) that at 10–15 cm depths the addition of digestate accelerated Ure hydrolysis more than in topsoil, putatively due to the retarded formation of nitrates.

Positive effects of digestate on both Ala-SIR and Man-SIR compared to unamended topsoil (20 and 40 $m^3 \cdot ha^{-1}$) and soil at depths of 10–15 cm (20 $m^3 \cdot ha^{-1}$) were revealed, also, in 2020. Again, a strong indirect dependence of Ala-SIR and Man-SIR on soil depth (declining in deeper layers) was detectable for the digestate-amended treatments. Therefore, we presumed a partial stabilization of introduced EOM in deeper soil layers of chernozem, together with immobilization of nitrogen, as was reported by other authors [44,45], and we assumed this from results of respiration and enzyme (ARS, NAG, and Ure) determinations. We verified hypothesis IV with these findings.

5. Conclusions

We found out that strip-till treatment of soil combined with the addition of digestate at three different depths (10, 15, and 20 cm) improved soil biological properties and microbial activity related to nutrient transformation, but differently in various seasons (2020 and 2021), due to differences in the properties of the applied digestate. We can generalize that the most significant improvements in oxidizable carbon, arylsulfatase, urease, and all respiration types in 2020 (compared to topsoil (0–10 cm)) was caused by the lower (20 $m^3 \cdot ha^{-1}$) digestate dose in mid-soil (10–15 cm). The most significant positive effects on arylsulfatase, N-acetyl-β-glucosaminidase, and all respiration types in 2021 were caused by higher (40 $m^3 \cdot ha^{-1}$) digestate doses in mid-soil (10–15 cm) in comparison to topsoil (0–10 cm). Moreover, strip-till-mediated application of digestate (20 or 40 $m^3 \cdot ha^{-1}$) in deeper soil layers (10–15 and 15–20 cm) mitigated depth-dependent decline in Cox (in 2020 and 2021) and β-glucosidase (in 2021) values (compared to corresponding layers of control unamended soil). We conclude that, under amendment with less rich digestate in the 2020 season, the impact of strip-till in combination with digestate amendment was less beneficial and more strongly negatively affected by soil depth, especially in relation to respiration indicators. On the contrary, in 2021, markedly positive impacts of digestate applied via strip-till were comparable for all three different depths of soil and thus verified the benefit of this agromanagement technique.

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References

1. Liu, D.; Huang, Y.; An, S.; Sun, H.; Bhople, P.; Chen, Z. Soil physicochemical and microbial characteristics of contrasting land-use types along soil depth gradients. *Catena* **2018**, *162*, 345–353. [CrossRef]
2. Srivastava, A.K.; Velmourougane, K.; Bhattacharyya, T.; Sarkar, D.; Pal, D.K.; Prasad, J.; Sidhu, G.S.; Nair, K.M.; Sahoo, A.K.; Das, T.H.; et al. Impacts of agro-climates and land use systems on culturable microbial population in soils of the Indo-Gangetic Plains, India. *Curr. Sci.* **2014**, *107*, 1464–1469.
3. Shrestha, R.K.; Lal, R. Land use impacts on physical properties of 28 years old reclaimed mine soils in Ohio. *Plant Soil* **2008**, *306*, 249–260. [CrossRef]
4. Reza, S.K.; Baruah, U.; Nayak, D.C.; Dutta, D.; Singh, S.K. Effects of Land-use on Soil Physical, Chemical and Microbial Properties in Humid Subtropical Northeastern India. *Natl. Acad. Sci. Lett.* **2018**, *41*, 141–145. [CrossRef]
5. Celik, I.; Barut, Z.B.; Ortas, I.; Gok, M.; Demirbas, A.; Tulun, Y.; Akpinar, C. Impacts of different tillage practices on some soil microbiological properties and crop yield under semi-arid Mediterranean conditions. *Int. J. Plant Prod.* **2011**, *5*, 237–254.
6. Miura, F.; Nakamoto, T.; Kaneda, S.; Okano, S.; Nakajima, M.; Murakami, T. Dynamics of soil biota at different depths under two contrasting tillage practices. *Soil Biol. Biochem.* **2008**, *40*, 406–414. [CrossRef]
7. Sun, B.; Jia, S.; Zhang, S.; McLaughlin, N.B.; Zhang, X.; Liang, A.; Chen, X.; Wei, S.; Liu, S. Tillage, seasonal and depths effects on soil microbial properties in black soil of Northeast China. *Soil Tillage Res.* **2016**, *155*, 421–428. [CrossRef]
8. Yadav, D.; Rani, K.; Wati, L. Impact of tillage practices on physico-chemical and microbiological properties of soil in wheat-pearl-millet cropping system. *Range Manag. Agrofor.* **2020**, *41*, 276–283.
9. Lavrenko, S.O.; Lavrenko, N.M.; Maksymov, D.O.; Maksymov, M.V.; Didenko, N.O.; Islam, K.R. Variable tillage depth and chemical fertilization impact on irrigated common beans and soil physical properties. *Soil Tillage Res.* **2021**, *212*, 105024. [CrossRef]
10. Vrtilek, P.; Smutny, V.; Neudert, L. Evaluation of the Impact of Different Soil Tillage on Physical Soil Properties. In Proceedings of the 24th International PhD Students Conference for Undergraduate and Postgraduate Students (MendelNet), Mendel Univ Brno, Fac AgriSciences, Brno, Czech Republic, 8–9 November 2017; pp. 164–168.
11. Álvaro-Fuentes, J.; Morell, F.J.; Madejón, E.; Lampurlanés, J.; Arrúe, J.L.; Cantero-Martínez, C. Soil biochemical properties in a semiarid Mediterranean agroecosystem as affected by long-term tillage and N fertilization. *Soil Tillage Res.* **2013**, *129*, 69–74. [CrossRef]
12. Mallory, J.J.; Mohtar, R.H.; Heathman, G.C.; Schulze, D.G.; Braudeau, E. Evaluating the effect of tillage on soil structural properties using the pedostructure concept. *Geoderma* **2011**, *163*, 141–149. [CrossRef]
13. Helgason, B.L.; Walley, F.L.; Germida, J.J. Fungal and Bacterial Abundance in Long-Term No-Till and Intensive-Till Soils of the Northern Great Plains. *Soil Sci. Soc. Am. J.* **2009**, *73*, 120–127. [CrossRef]
14. van Groenigen, K.-J.; Bloem, J.; Bååth, E.; Boeckx, P.; Rousk, J.; Bodé, S.; Forristal, D.; Jones, M.B. Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. *Soil Biol. Biochem.* **2010**, *42*, 48–55. [CrossRef]
15. Godsey, C.; Kochenower, R.; Taylor, R. Strip-till Considerations in Oklahoma. *Okla. Coop. Ext. Serv.* **2017**, *2*, PSS-2134.
16. Bakhitova, A.R.; Mamontov, V.G.; Enakiev, Y.I.; Stefanov, K.G. Variability of Soil Nutritional Parameters in Small-plot Experiment with Mineral Fertilizers Application in Different Depth. In Proceedings of the 7th International Conference on Energy Efficiency and Agricultural Engineering (EE and AE), Ruse, Bulgaria, 12–14 November 2020.
17. Ozdemir, N.; Yakupoglu, T.; Dengiz, O. The effects of bio-solid and tea waste application into different levels of eroded soil on N, P and K concentrations. *Environ. Monit. Assess.* **2008**, *156*, 109. [CrossRef]

18. USDA. Conservation practice definitions and their corresponding potential to adversely impact cultural resources. *Nat. Resour. Conserv. Serv.* **2013**, 11.
19. Koszel, M.; Lorencowicz, E. Agricultural Use of Biogas Digestate as a Replacement Fertilizers. *Agric. Agric. Sci. Procedia* **2015**, *7*, 119–124. [CrossRef]
20. Slepetiene, A.; Volungevicius, J.; Jurgutis, L.; Liaudanskiene, I.; Amaleviciute-Volunge, K.; Slepetys, J.; Ceseviciene, J. The potential of digestate as a biofertilizer in eroded soils of Lithuania. *Waste Manag.* **2020**, *102*, 441–451. [CrossRef]
21. *ISO_14235:1998*; Soil quality—Determination of organic carbon by sulfochromic oxidation. International Organization for Standardization: Geneva, Switzerland, 1998.
22. *ISO_20130:2018*; Soil quality—Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates. International Organization for Standardization: Geneva, Switzerland, 2018.
23. Campbell, C.D.; Chapman, S.J.; Cameron, C.M.; Davidson, M.S.; Potts, J.M. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Appl. Environ. Microbiol.* **2003**, *69*, 3593–3599. [CrossRef]
24. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
25. Duffkova, R.; Macurova, H. Soil biological quantity and quality parameters of grasslands in various landscape zones. *Plant Soil Environ.* **2011**, *57*, 577–582. [CrossRef]
26. Diederich, K.M.; Ruark, M.D.; Krishnan, K.; Arriaga, F.J.; Silva, E.M. Increasing Labile Soil Carbon and Nitrogen Fractions Require a Change in System, Rather Than Practice. *Soil Sci. Soc. Am. J.* **2019**, *83*, 1733–1745. [CrossRef]
27. Ma, X.Z.; Chen, L.J.; Chen, Z.H.; Wu, Z.J.; Zhang, L.L.; Zhang, Y.L. Soil glycosidase activities and water soluble organic carbon under different land use types. *Rev. Cienc. Suelo Y Nutr. Veg.* **2010**, *10*, 93–101. [CrossRef]
28. Pereira, A.A.; Borges, A.C.; Matos, M.P.; Diniz, I.C.C.; Matos, A.T. Degradation rate of anaerobically digested sewage sludge in soil. *J. Water Sanit. Hyg. Dev.* **2018**, *8*, 17–26. [CrossRef]
29. Musatti, A.; Ficara, E.; Mapelli, C.; Sambusiti, C.; Rollini, M. Use of solid digestate for lignocellulolytic enzymes production through submerged fungal fermentation. *J. Environ. Manag.* **2017**, *199*, 1–6. [CrossRef] [PubMed]
30. Plaza, C.; Hernández, D.; García-Gil, J.C.; Polo, A. Microbial activity in pig slurry-amended soils under semiarid conditions. *Soil Biol. Biochem.* **2004**, *36*, 1577–1585. [CrossRef]
31. Meyer, A.H.; Wooldridge, J.; Dames, J.F. Variation in urease and β-glucosidase activities with soil depth and root density in a 'Cripp's Pink'/M7 apple orchard under conventional and organic management. *S. Afr. J. Plant Soil* **2015**, *32*, 227–234. [CrossRef]
32. Tabatabai, M.; Bremner, J. Factors affecting soil arylsulfatase activity. *Soil Sci. Soc. Am. J.* **1970**, *34*, 427–429. [CrossRef]
33. Deng, S.P.; Tabatabai, M.A. Effect of tillage and residue management on enzyme activities in soils: III. Phosphatases and arylsulfatase. *Biol. Fertil. Soils* **1997**, *24*, 141–146. [CrossRef]
34. Balota, E.L.; Machineski, O.; Truber, P.V. Soil enzyme activities under pig slurry addition and different tillage systems. *Acta Scientiarum. Agron.* **2011**, *33*, 729–737. [CrossRef]
35. Lalande, R.; Gagnon, B.; Simard, R.R.; Côté, D. Soil microbial biomass and enzyme activity following liquid hog manure application in a long-term field trial. *Can. J. Soil Sci.* **2000**, *80*, 263–269. [CrossRef]
36. García-Sánchez, M.; Siles, J.A.; Cajthaml, T.; García-Romera, I.; Tlustoš, P.; Száková, J. Effect of digestate and fly ash applications on soil functional properties and microbial communities. *Eur. J. Soil Biol.* **2015**, *71*, 1–12. [CrossRef]
37. Li, N.; Xu, Y.-Z.; Han, X.-Z.; He, H.-B.; Zhang, X.-d.; Zhang, B. Fungi contribute more than bacteria to soil organic matter through necromass accumulation under different agricultural practices during the early pedogenesis of a Mollisol. *Eur. J. Soil Biol.* **2015**, *67*, 51–58. [CrossRef]
38. Wang, L.L.; Li, W.Z.; Wang, Z.J.; Wang, Z.W.; Sui, C.; Li, Y. Effects of digestate application depth on soil nitrogen volatilization and vertical distribution. *Int. J. Agric. Biol. Eng.* **2016**, *9*, 101–107. [CrossRef]
39. Hu, X.; Liu, H.; Xu, C.; Huang, X.; Jiang, M.; Zhuang, H.; Huang, L. Effect of Digestate and Straw Combined Application on Maintaining Rice Production and Paddy Environment. *Int. J. Environ. Res. Public Health* **2021**, *18*, 5714. [CrossRef] [PubMed]
40. Elbl, J.; Simeckova, J.; Skarpa, P.; Kintl, A.; Brtnicky, M.; Vaverkova, M.D. Comparison of the Agricultural Use of Products from Organic Waste Processing with Conventional Mineral Fertilizer: Potential Effects on Mineral Nitrogen Leaching and Soil Quality. *Agronomy* **2020**, *10*, 226. [CrossRef]
41. Habova, M.; Vlcek, V.; Simeckova, J.; Hybler, V.; Pospisilova, L.; Jandak, J. Response of microbial associations to fertilizers application. In Proceedings of the 23rd International PhD Students Conference (MendelNet), Mendel Univ Brno, Fac AgriSciences, Brno, Czech Republic, 9–10 November 2016; pp. 411–416.
42. Fang, C.; Moncrieff, J.B. The variation of soil microbial respiration with depth in relation to soil carbon composition. *Plant Soil* **2005**, *268*, 243–253. [CrossRef]
43. Spohn, M.; Chodak, M. Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. *Soil Biol. Biochem.* **2015**, *81*, 128–133. [CrossRef]

44. Horta, C.; Carneiro, J.P. Use of Digestate as Organic Amendment and Source of Nitrogen to Vegetable Crops. *Appl. Sci.* **2021**, *12*, 248. [CrossRef]
45. Greenberg, I.; Kaiser, M.; Gunina, A.; Ledesma, P.; Polifka, S.; Wiedner, K.; Mueller, C.W.; Glaser, B.; Ludwig, B. Substitution of mineral fertilizers with biogas digestate plus biochar increases physically stabilized soil carbon but not crop biomass in a field trial. *Sci Total Environ.* **2019**, *680*, 181–189. [CrossRef]