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Effects of Biogas Digestate on Winter Wheat Yield, Nitrogen Balance, and Nitrous Oxide Emissions under Organic Farming Conditions

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Abstract: Biogas digestate is increasingly used in organic farming to improve soil nutrient supply and sustainably increase yields. However, biogas digestate can also lead to environmentally relevant N_2O emissions. The benefits, opportunities, and risks associated with the use of digestate as a fertilizer in organic farming are a subject of ongoing debate, in part due to a lack of conclusive experimental results. A field trial conducted in southern Germany examined the short-term and long-term impacts of digestate fertilization on winter wheat yield, nitrogen use efficiency, and N_2O-N emissions. The four-year results from the years 2019 to 2022 are presented. Digestate was applied with a nitrogen input of up to 265 kg ha^{-1}, with 129 kg ha^{-1} $NH_4{}^+$-N. The application of digestate resulted in a significant increase in wheat yield, with an average increase of 53% (2019) to 83% (2022) compared to the unfertilized control. It is notable that the treatment applied for the first time did not reach the yield of the long-term fertilized treatment, with a yield gap of 0.5 to 1.2 Mg ha^{-1} (6% to 15%). The highest N_2O-N emissions (up to 3.30 kg ha^{-1}) in the vegetation period from spring to autumn were measured in the long-term fertilized treatment. However, very high N_2O-N emissions (up to 3.72 kg ha^{-1}) also occurred in two years in winter in the unfertilized treatment. An increase in soil inorganic N stocks and N_2O-N emissions was observed following the wheat harvest and subsequent tillage in all treatments. No significant differences were identified between the fertilizer treatments with regard to product-related emissions. The experimental results demonstrate that N_2O-N emissions are not solely a consequence of N fertilization, but can also be attributed to tillage, post-harvest practices, and previous crops, with considerable variability depending on weather conditions. The experimental data provide comprehensive insight into the influence of cultivation, soil characteristics, and meteorological conditions on N_2O-N emissions at an agricultural site in southern Germany.

Keywords: N_2O emission; nitrogen turnover; digestate; greenhouse gas; organic farming; long-term experiment; crop yield

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1. Introduction

Biogas production from energy crops and manure has been greatly expanded over the last 20 years [1,2]. The use of biomass fermentation is primarily intended to conserve fossil fuel resources and reduce greenhouse gas emissions [3,4]. This approach is only viable if the emissions from the production and energetic use of biomass are significantly lower than those produced by fossil fuels [5]. The nitrous oxide (N_2O) fluxes caused by the utilization of fermentation residues must therefore be known in order to calculate greenhouse gas balances [6,7]. With the rapid expansion of biogas plants, large amounts of biogas digestate are produced. Biogas digestates contain valuable macro- and micronutrients and organic matter, which can be used as fertilizers or soil amendments [8–10].

N_2O is a long-lived greenhouse gas with a current atmospheric concentration of 337.7 ppb [11]. The CO_2 equivalent of N_2O is 298, based on a 100-year investigation period [12]. The formation of N_2O is a natural process and part of the nitrogen (N) cycle.

N_2O is formed mostly by microorganism during the conversion of ammonium (NH_4^+) by nitrification and nitrate (NO_3^-) by denitrification [13]. The N_2O emissions depend on numerous natural factors such as soil and weather conditions as well as management factors such as crop rotation, fertilizer quantity and composition, application methods and timing, and tillage intensity [14,15]. Fertilization with biogas digestate inevitably leads to N_2O emissions [16], due to the supply of C and N compounds. However, the interactions between the above-mentioned factors under field conditions have not yet been sufficiently clarified [17,18]; the level of N_2O fluxes after digestate application can vary greatly and is difficult to estimate [19–21].

Biogas digestate is increasingly being used in organic farming to improve the soil nutrient supply and sustainably increase crop yields [22–26]. The production methods of organic farming are defined in EU regulations [27]; these rules also form the basis of our experiment. The benefits, opportunities, and risks of the use of digestate in organic agriculture are assessed controversially [28]. So far, there are hardly any results on yields, N balances, and N losses, e.g., N_2O emissions from field trials with digestates in organic farming. The results from conventional field trials show comparable or even higher yields with digestate than with unfermented slurry [23,29,30]. In organically grown winter wheat, high yield increases were found with digestate application (up to over 50% compared to non-fertilized or green manure-fertilized treatments) [22]. Of particular importance for the yield effects of biogas digestate is the increase in plant-available inorganic N due to the decomposition of organic matter during the fermentation process, which can lead to a 33% higher NH_4^+ content compared to unfermented slurry [28,31–33]. About 44–81% of the total N of biogas digestate is in the form of NH_4^+ [31,34].

Previous studies on yield effects and N_2O emissions from digestate often only refer to short-term effects or were carried out under laboratory conditions [17,18,35]. Until now, there has been a lack of long-term field experiments in which the effects of digestate could be analyzed under organic farming conditions. Therefore, the accumulative effects of digestate on the soil C and N turnover, on nitrification and denitrification processes, and on long-term yield effects could not yet be assessed on the basis of experimental data [36].

Years of continuous fertilization with biogas digestates, as is common for farms with biogas plants, add large amounts of organic C and N to the soils, which has complex effects on soils, plants, and the environment [37]. Thus, soil organic C (SOC) and soil total N (TN) contents, C and N turnover, denitrification, and N_2O emissions could increase with duration of digestate use. However, it has not been investigated if the continuous application of biogas digestate leads to higher N_2O fluxes than a single application [28]. The long-term effects of digestate on crop yields are also unclear. It is possible that the yield potential will increase with the continuous application of digestate. Therefore, only a long-term experiment with a sufficiently long test duration can provide reliable information on the product-related N_2O emissions.

In order to close the knowledge gap described above and to gain new insights into the short- and long-term effects of digestate, analyses of the effects on crop yield, N efficiency, and N_2O-N emissions were carried out in a long-term field experiment. Four-year experimental results from 2019 to 2022 are presented for the crop winter wheat. The experimental basis is the Viehhausen energy crop rotation trial in southern Germany, where the effects of fertilization with digestate have been investigated since 2005.

The objectives of the study are to assess the dynamics of N_2O emissions over an extended period and to evaluate the influence of soil properties, agronomic practices, and environmental factors on N_2O emissions. Additionally, the study aimed to rate yield and product quality, N balances, and product-related emissions in organic farming systems from an agronomic perspective. It also sought to provide insights into the potential of biogas digestate in promoting sustainable agriculture.

2. Materials and Methods

2.1. Site and Weather Conditions

The field trial was conducted at the Viehhausen Experimental Station of the Technical University of Munich, which is located near Freising, 30 km north of Munich (48°39′62′54 N, 11°65′07′31 E), 490 m above sea level in the Bavarian Tertiary Hills. The soil of the experiment is a Haplic Luvisol, composed of loess, with 25% clay, 62% silt, and 13% sand (silty loam). The soil has a high water-holding capacity due to its high clay content and tends to compaction and sludging. The chemical soil properties are listed separately for long-term fertilized and unfertilized treatments in Table 1 (note that plots rotate with the crop rotation).

Table 1. Soil properties at 0–30 cm depth, long-term trial "energy crop rotation", experimental station Viehhausen. Investigated plots rotate with the crop rotation.

Parameter	Unit	2019		2020		2021		2022	
		00 [a]	DD [b]	00	DD	00	DD	00	DD
SOC	%	0.94	1.10	0.83	1.02	1.03	1.11	0.90	1.20
TN	%	0.11	0.13	0.10	0.12	0.11	0.12	0.11	0.15
C/N		8.6	8.5	8.7	8.6	9.2	9.1	8.2	8.0
pH		6.1	6.2	6.3	6.4	6.0	6.1	5.8	5.9
CAL-P [c]	mg 100 g^{-1}	1.04	1.53	2.33	3.93	2.07	2.87	1.25	1.80
CAL-K [d]	mg 100 g^{-1}	6.03	10.92	6.09	12.33	5.53	9.98	5.72	8.62
Cmic [e]	mg kg^{-1}	427	491	422	523	492	618	521	588
Bulk density	g cm^{-3}	1.31	1.27	1.40	1.40	1.42	1.46	1.30	1.31

[a]: long-term unfertilized treatment, [b]: long-term fertilized treatment, [c]: calcium–acetate–lactate phosphorus, [d]: calcium–acetate–lactate potassium, [e]: microbial biomass.

The long-term means (1991–2020) of temperature and precipitation at this site are 8.7 °C and 774 mm per year, respectively (Table 2). In all four years of the study (2019 to 2022), it was warmer than the long-term average. The years were characterized by a dry and warm spring, especially in March and April, followed by periods of heavy rainfalls in summer. May was wet in 2019 and 2021 but rather dry in 2020 and 2022. Both summer and autumn were warm compared to the long-term mean. Winters were very mild compared to the long-term average, with few frost days. The mean temperatures in 2019, 2020, and 2022 were above the long-term average, while 2021 had slightly lower temperatures. The mean precipitation in the experimental years 2019, 2020, and 2022 was found to be consistent with the long-term average. However, the precipitation in 2021 was considerably higher.

Table 2. Average monthly temperature and precipitation for the years 2019–2022 and the long-term average (1961–1990 and 1991–2020), experimental station Weihenstephan-Dürnast (3 km from Viehhausen).

	Unit	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean
1961–1990														
Temperature	[°C]	−2.1	−0.7	3.0	7.3	11.9	15.0	16.7	16.1	12.9	7.9	2.8	−0.7	7.5
Precipitation	[mm]	43.9	38.9	43.4	55.5	89.6	103.7	98.3	97.1	64.2	50.5	54.0	48.4	787.7
1991–2020														
Temperature	[°C]	−0.6	0.4	4.5	8.8	13.0	16.3	18.0	18.0	13.2	8.7	3.8	0.5	8.7
Precipitation	[mm]	44.7	34.0	46.8	42.5	85.8	99.1	98.4	87.1	67.4	60.6	52.9	54.7	774.2
2019														
Temperature	[°C]	−0.6	2.2	6.3	10.1	10.6	19.6	19.0	18.7	13.8	10.2	4.5	2.2	9.8
Precipitation	[mm]	86.3	38.3	48.5	12.3	118	79.3	54.3	98.9	48.4	50.2	35.9	1.1	726.8

Table 2. *Cont.*

	Unit	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean
2020														
Temperature	[°C]	1.3	4.7	5.0	10.6	11.8	16.1	18.5	18.8	14.3	9.0	4.2	1.2	9.6
Precipitation	[mm]	25.9	96.0	37.5	23.4	31.8	154.1	54.0	104.3	73.7	90.0	16.4	45.7	766.8
2021														
Temperature	[°C]	−0.7	2.3	4.1	6.3	10.5	18.8	17.9	16.4	14.5	8.0	2.8	2.0	8.6
Precipitation	[mm]	53.7	40.2	36.6	29.0	161.0	131.0	114.8	166.7	35.9	17.7	36.9	9.0	914.4
2022														
Temperature	[°C]	1.2	3.8	4.6	7.4	14.8	18.8	19.6	19.4	12.7	11.9	5.2	1.3	10.1
Precipitation	[mm]	48.5	36.7	13.8	57.4	70.1	99.4	57.0	85.4	78.3	60.6	55.9	56.6	719.7

2.2. Experimental Set Up

The field trial is part of the long-term field experiment "energy crop rotation trial". This field experiment was established in 2004 and covers an area of 3.75 ha. It consists of 384 plots and contains different energy and cash crops, crop rotations, and fertilization and tillage systems [38,39]. In this study, only the long-term fertilized and unfertilized treatments of winter wheat, grown in the crop rotation clover/grass–winter wheat–maize–triticale, was investigated. The long-term fertilized and unfertilized plots were subdivided at the time of fertilization, resulting in the following four treatments (Table 3): unfertilized control treatment (00), treatment that was fertilized with digestate in the long term (DD), treatment fertilized with digestate for the first time (0D, analysis of the direct effects of digestate), and treatment not fertilized with digestate for the first time (D0, analysis of the after-effect of biogas digestate). The experimental design is a single-factor randomized block unit [38]. Each plot has a size of 72 m^2 (12 m × 6 m), after dividing 36 m^2 (12 m × 3 m). For the determination of the yield parameters and N balancing, 4 treatments with 4 replicates (=16 plots) were used, and for the N_2O measurements, 3 replicates (=12 plots) were used.

Table 3. Abbreviations of the treatments and explanations.

Treatment *	Previous Years (2004–2018)	Years of Study (2019–2022)	Explanation
00	Unfertilized	Unfertilized	Control treatment
D0	Digestate	Unfertilized	Analysis of the after-effects of digestate
0D	Unfertilized	Digestate	Analysis of the direct effects of digestate
DD	Digestate	Digestate	Analysis of the long-term effects of digestate

* Treatments rotate with crop rotation of the long-term trial. Treatments D0 and 0D were only treated once in the year of measurement. Treatments 00 and DD have had unchanged treatment since 2004.

2.3. Fertilization with Biogas Digestate

The N rates of digestate applied in the trial correspond to the digestate production derived from the biomass yield of the energy crops and was in the range of 223.2 and 264.6 kg ha^{-1}. The N supply with digestate did not exceed the fertilization requirement according to the German Fertilizer Ordinance [40]. Digestate was applied using a slurry tanker with trailing hoses at two dates each year—at the beginning of vegetation (BBCH 20/21) and at growth stage BBCH 30/31 (Table A1) [41]. The biogas digestate was produced by a local organic farmer from a feedstock mixture corresponding to the biomass produced in the trial (clover–grass and maize silage). The chemical composition of the digestate is summarized in Table 4.

Table 4. Chemical composition of the biogas digestate applied to winter wheat in 2019–2022.

Parameter	Unit	2019	2020	2021	2022
Dry matter (DM)	%	9.55	9.20	8.30	8.65
Tot-C	% DM	38.85	38.70	42.03	40.85
Tot-N	% DM	6.11	7.19	6.89	6.52
NH_4-N	% Tot-N	41.73	48.68	52.25	50.65
C:N		6.36	5.38	6.10	5.95
K_2O-K	% DM	8.02	8.82	9.93	9.47
P_2O_5-P	% DM	2.00	1.99	2.31	2.54
Tot-S	% DM	0.51	0.49	0.55	0.54

2.4. Biomass and Soil Samples

For grain yield determination, the plots were harvested with a plot combine. Crop samples were dried at 105 °C. Dry matter yield was determined and extrapolated to one hectare. The determination of the N and C contents of the soil and the plant biomass was carried out with an Elementar vario MAX C/N analyzer [42]. The wheat grain protein content was calculated from the N content multiplied by 5.7 [43].

For the analysis of inorganic N content, soil samples were taken weekly with a soil auger at a soil depth of 0–15 cm and 15–30 cm. A soil sample consisted of a composite sample of four replicates. The soil samples were homogenized and then solved in 0.1 mol/L KCl extract to determine the parameters NO_3^- and NH_4^+ [44]. The NO_3^--N and NH_4^+-N stocks were determined for the soil layers using the specific bulk density. The gravimetric water content was determined by drying at 105 °C in a compartment drier.

N balances were calculated to evaluate the N surplus (corresponds to the total loss potential of reactive N compounds) and the N use efficiency (NUE).

$$N\ surplus = Ninput - N\ uptake \quad (1)$$

The N grain uptake corresponds to the dry matter yield multiplied by the N content of the grain. The total N uptake represents the N uptake of grain and straw.

The N surplus was calculated as the difference between fertilizer N input (total N of the digestate) and total N uptake. The NUE results from the N uptake in relation to the N input [45].

$$NUE = \frac{N\ uptake}{N\ input} \times 100 \quad (2)$$

2.5. N_2O Measurement

Gas samples were collected using the manual closed chamber method [46] according to the guidelines of Nitrous Oxide Chamber Methodology [47]. Chambers were a special design by the company Ps Plastic (Eching, Germany). Gas samples were taken once a week and additionally in periods where high N_2O flux rates were expected (after fertilization, after heavy rainfalls, after tillage, or in frost–thaw cycles) to obtain a high resolution of trace gas fluxes and to avoid under- or overestimation of cumulative N_2O emissions. In order to be able to cumulate the N_2O fluxes, the measurements were carried out between 08:00 and 11:00 to cover the mean daily temperature [48] and best approximated daily mean N_2O flux [49]. The chambers had an area of 0.36 m^2 and were equipped with a rubber seal, a fan, and a degassing hose. For the measurements, the chamber was placed over the vegetation on a frame fixed into the soil and left there for an hour. Samples were taken with a battery-operated sampler in glass vials after 0, 20, 40, and 60 min. The vials, sealed with a septum, were analyzed by a gas chromatograph provided with an electron capture detector (ECD). The calibration range was 300 to 3000 ppb for N_2O.

The actual flow rate was calculated with the statistic software RStudio (Version 1.3.1093) and the package "gasfluxes" [50] for each chamber based on the increase in the gas concentration in the chambers over time (1 h and 4 measurements) and expressed in ppm. In consideration of the specific chamber temperature, the nitrous oxide fluxes were converted into the unit µg m^{-2} h^{-1} and are presented as N_2O-N emissions.

2.6. Calculation of Cumulative N_2O Emissions

Linear interpolation between two measurement events was used to cumulate N_2O fluxes [17,20]. In order to identify influences of seasons and vegetation periods, the cumulated emissions were recorded for different periods (whole year, winter, summer, and post-harvest). Autumn and winter emissions were only measured in the two long-term treatments in all years, as the other treatments only emerged after fertilization. To calculate the area-related and product-related emissions, fluxes of the long-term treatments were added on the first-time treatments.

For a better representation of the statistical differences in N_2O-N fluxes, the data were manipulated to obtain negative fluxes out (N_2O-N + 50 µg m^{-2} h^{-1}). Product-related emissions were calculated by N_2O-N emissions per hectare divided by wheat yield per hectare [51]. The emission factor was calculated by subtracting the cumulative emissions of the fertilized treatment ($Flux_{fertilized}$) from the emissions of the unfertilized control treatment ($Flux_{control}$) in relation to the N input) [52–54].

$$EF = \frac{Flux_{fertilized} - Flux_{control}}{N\ input} \times 100 \quad (3)$$

In addition, the Global Nitrous Oxide Calculator (GNOC) was used, a model based on an exponential algorithm that considers site- and management-specific characteristics such as soil texture, climate, soil organic matter, pH, and vegetation [55].

2.7. Statistical Analysis

The statistics software RStudio (Version 1.3.1093) was used for the statistical evaluation. A linear mixed model and ANOVA at a significance level of α = 0.05 were performed with the packages "lme4" and "lmerTest" [56]. Treatment and year were set as fixed factors and blocks and replicates as random factors. A statistical comparison of the cumulated fluxes was carried out by logarithmic transformation to improve variance homogeneity and subsequent evaluation with a linear mixed-effects model. Furthermore, all test factors were evaluated with the post hoc test "Tukey" to detect significant differences between the treatments (significance level of α = 0.05). Significant differences are represented by superscript letters.

3. Results

3.1. Grain Yield and Protein Content

Wheat grain yields ranged from 3.8 Mg ha^{-1} (treatment 00, long-term unfertilized, 2020) to 8.4 Mg ha^{-1} (treatment DD, long-term fertilized with digestate, 2022) (Table 5). In all test years, treatment DD achieved the highest yields and treatment 00 the lowest. A significant after-effect of digestate fertilization on wheat yield was found in 2019 and 2022 (comparison of treatments D0 and 00). In 2019, treatment 0D, which was fertilized with digestate for the first time, achieved a yield of 6.9 Mg ha^{-1}, which did not differ significantly from treatment D0 (6.2 Mg ha^{-1}), but was significantly lower than in treatment DD (8.1 Mg ha^{-1}). In 2020 and 2021, the two fertilized treatments (0D and DD) and the two unfertilized treatments (00 and D0) were not significantly different in grain yield. In 2022, treatment 00 had by far the lowest yield with 4.6 Mg ha^{-1}. Treatment D0 had a significantly higher yield than treatment 00 and a significantly lower yield than the two fertilized treatments (0D and DD). The yields in all four years indicate an effect of long-term and first-time fertilization. The yields can be ranked in ascending order: 00 < D0 < 0D < DD.

Table 5. Wheat grain yield in fresh matter, standardized to 86% dry matter, protein content, N uptake, N surplus, and N use efficiency (NUE) for the years 2019–2022. Different letters indicate significant differences (Tukey test, $p < 0.05$).

Year	Treatment *	N Input kg ha^{-1}	NH_4^+-N Input kg ha^{-1}	Yield Mg ha^{-1}	Protein Content % DM	N Uptake kg ha^{-1}	N Surplus kg ha^{-1}	NUE %
2019	00	0	0	5.3 a	10.7 a	105.7 a	−105.7 a	
	D0	0	0	6.2 b	10.6 a	123.7 ab	−123.7 a	
	0D	233.4	97.4	6.9 b	10.7 a	138.1 b	95.3 c	59.2 a
	DD	233.4	97.4	8.1 c	11.4 a	170.0 c	63.4 b	72.8 b
2020	00	0	0	3.8 a	10.1 a	73.5 a	−73.5 a	
	D0	0	0	4.4 a	10.9 a	90.1 a	−90.1 a	
	0D	264.6	128.8	6.4 b	11.1 a	131.6 b	133.0 b	49.7 a
	DD	264.6	128.8	6.9 b	11.1 a	142.8 b	121.8 b	54.0 a
2021	00	0	0	5.0 a	10.0 a	95.0 a	−95.0 a	
	D0	0	0	6.0 a	10.2 a	116.3 ab	−116.3 a	
	0D	228.7	119.5	6.7 b	11.2 a	139.8 bc	89.0 b	61.1 a
	DD	228.7	119.5	7.7 b	10.9 a	157.5 c	71.2 b	68.9 a
2022	00	0	0	4.6 a	11.4 a	95.7 a	−95.7 a	
	D0	0	0	6.4 b	11.5 a	137.0 b	−137.0 a	
	0D	223.2	113.1	7.9 c	11.3 a	164.5 c	58.7 d	73.7 a
	DD	223.2	113.1	8.4 c	12.5 a	190.2 d	33.0 c	85.2 b

* Treatments: 00 = long-term unfertilized, D0 = first-time unfertilized, 0D = first-time fertilized, and DD = long-term fertilized.

The protein contents, considered for all years, were between 10.0 and 12.5%, which is average for organically grown winter wheat. No significant differences between the treatments were identified.

3.2. N Uptake, N Surplus, and N Use Efficiency

The high N inputs with digestate (223.3 to 264.6 kg ha^{-1}) resulted in a high N uptake (up to 190.2 kg ha^{-1}) (Table 5). In the unfertilized treatment 00, the N uptake ranged from 73.5 kg ha^{-1} to 105.7 kg ha^{-1}, which can be attributed to soil N mineralization and N transfer from the clover–grass.

Considering the measured NH_4^+-N stocks of the digestate, the supply of plant-available NH_4^+-N in the fertilized treatments was 97.4 to 128.8 kg ha^{-1}. There were significant differences in N uptake between the first-time and long-term treatments in all years except 2020 (in 2020, there were only significant differences between the fertilized and unfertilized treatments). It was observed that the long-term unfertilized treatment always resulted in a lower N uptake than the first-time unfertilized treatment, while the long-term fertilized treatment always had a higher N uptake than the first-time fertilized treatment. The N balances of the unfertilized treatments were negative (−73.5 to −137.0 kg ha^{-1}). This indicates a possible decrease in soil total N stocks. In fact, the soil N contents in the unfertilized treatment 00 were lower than those of the fertilized treatment DD (Table 1). The N balances of the long-term fertilized treatment DD ranged from 33.0 to 121.8 kg ha^{-1} and the N balances of the first-time fertilized treatment 0D from 58.7 to 133.0 kg ha^{-1}.

3.3. NH_4^+, NO_3^-, and Soil Moisture Dynamics

The dynamics of NH_4^+ and NO_3^- stocks in the soil, soil moisture, and N_2O-N emissions are shown in Figure 1 for wheat cultivation in 2019 from the start of the measurements on 26 March 2019 to the end of the measurements on 3 September 2019. In the following years, measurements started in October, shortly after winter wheat sowing. The measurement periods vary slightly from year to year, depending on the sowing and harvest dates.

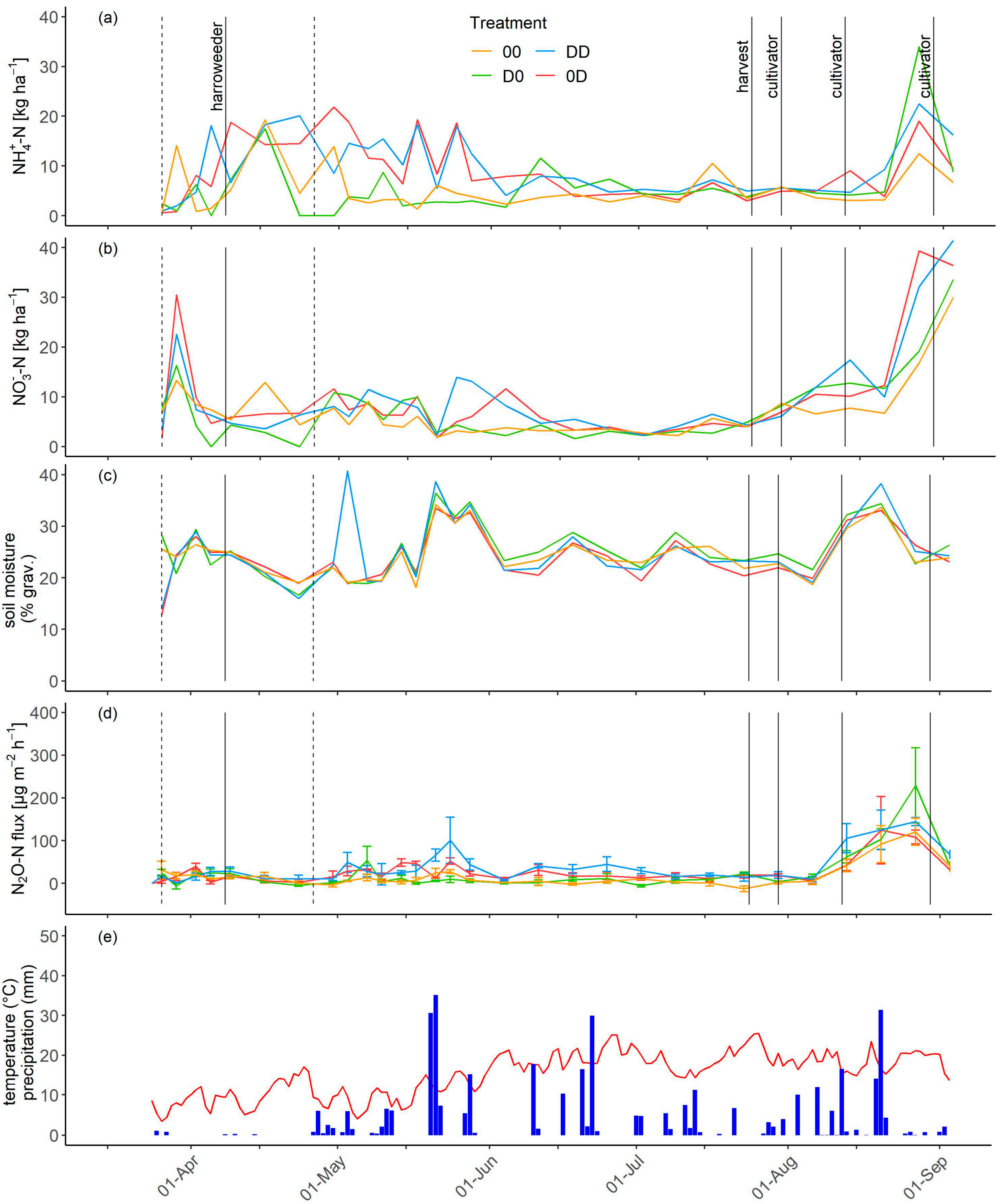

Figure 1. Ammonium-N (**a**) and nitrate-N dynamics (**b**) and soil moisture (**c**) of all four treatments related to the topsoil layer (0–15 cm) from March 2019 to September 2019. Nitrous oxide emissions (**d**), error bars illustrate the standard deviation) and temperature (red line) and precipitation (**e**) from March 2019 to September 2019. Black lines mark different agronomic actions and dashed lines mark the date of fertilization and only refer to the two fertilized treatments. (Treatments: 00 long-term unfertilized, D0 first-time unfertilized, 0D first-time fertilized, and DD long-term fertilized).

The NH_4^+ and NO_3^- stocks in the soil layer 0–15 cm were rather low throughout the trial period. Very low NH_4^+ and NO_3^--N stocks (<10 kg ha^{-1}) were observed over longer periods (especially in summer and winter). Increases of up to 34 kg ha^{-1} NH_4^+-N and 46 kg ha^{-1}

NO_3^--N were recorded in spring after fertilization and in late summer/autumn post-harvest. However, the NH_4^+ and NO_3^- dynamics were year- and weather-specific (Figures 1–4).

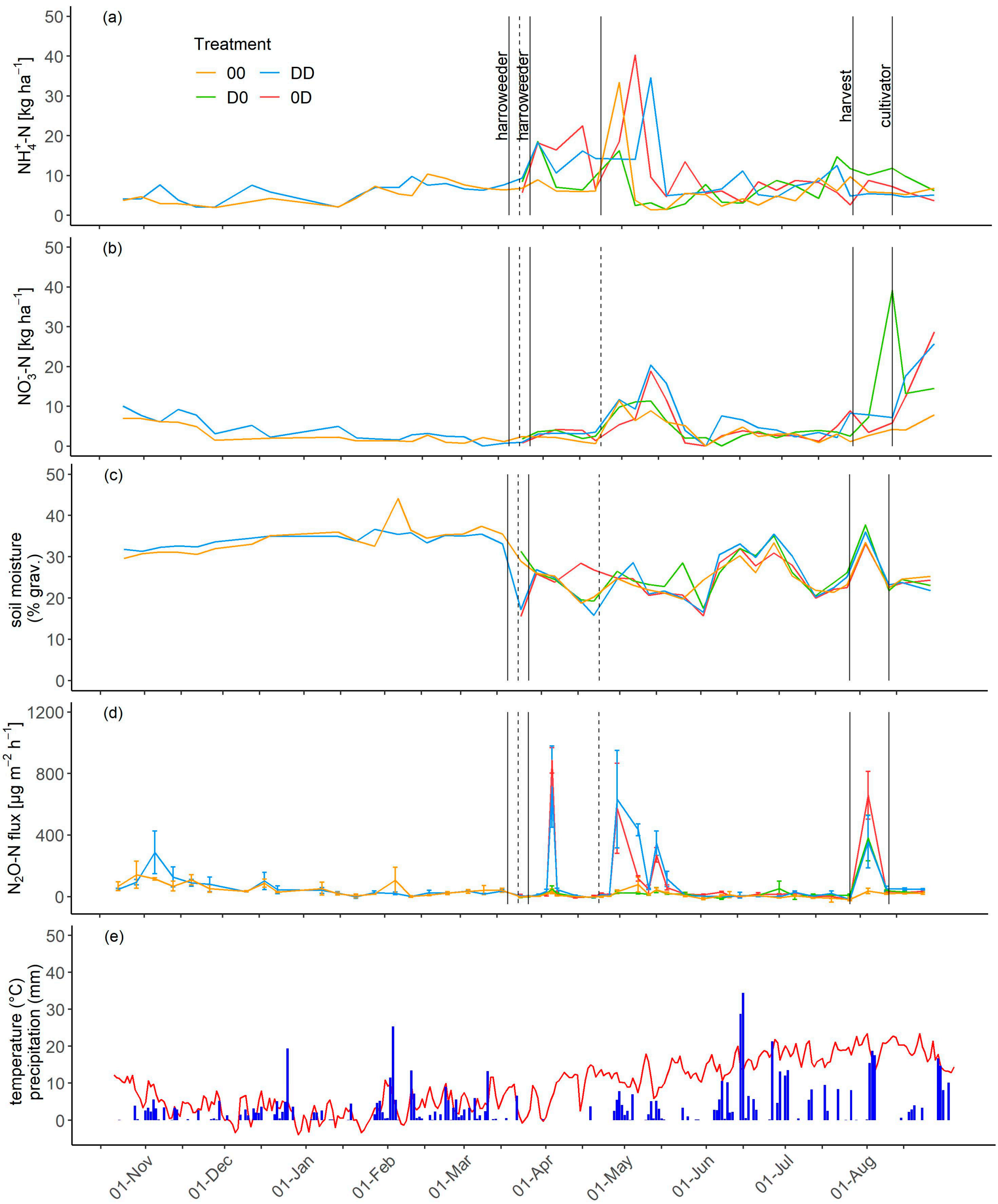

Figure 2. Ammonium-N (**a**) and nitrate-N dynamics (**b**) and soil moisture (**c**) of all four treatments related to the topsoil layer (0–15 cm) from October 2019 to September 2020. Nitrous oxide emissions (**d**), error bars illustrate the standard deviation) and temperature (red line) and precipitation (**e**) from October 2019 to September 2020. Black lines mark different agronomic actions and dashed lines mark the date of fertilization and only refer to the two fertilized treatments (DD and 0D). (Treatments: 00 long-term unfertilized, D0 first-time unfertilized, 0D first-time fertilized, and DD long-term fertilized).

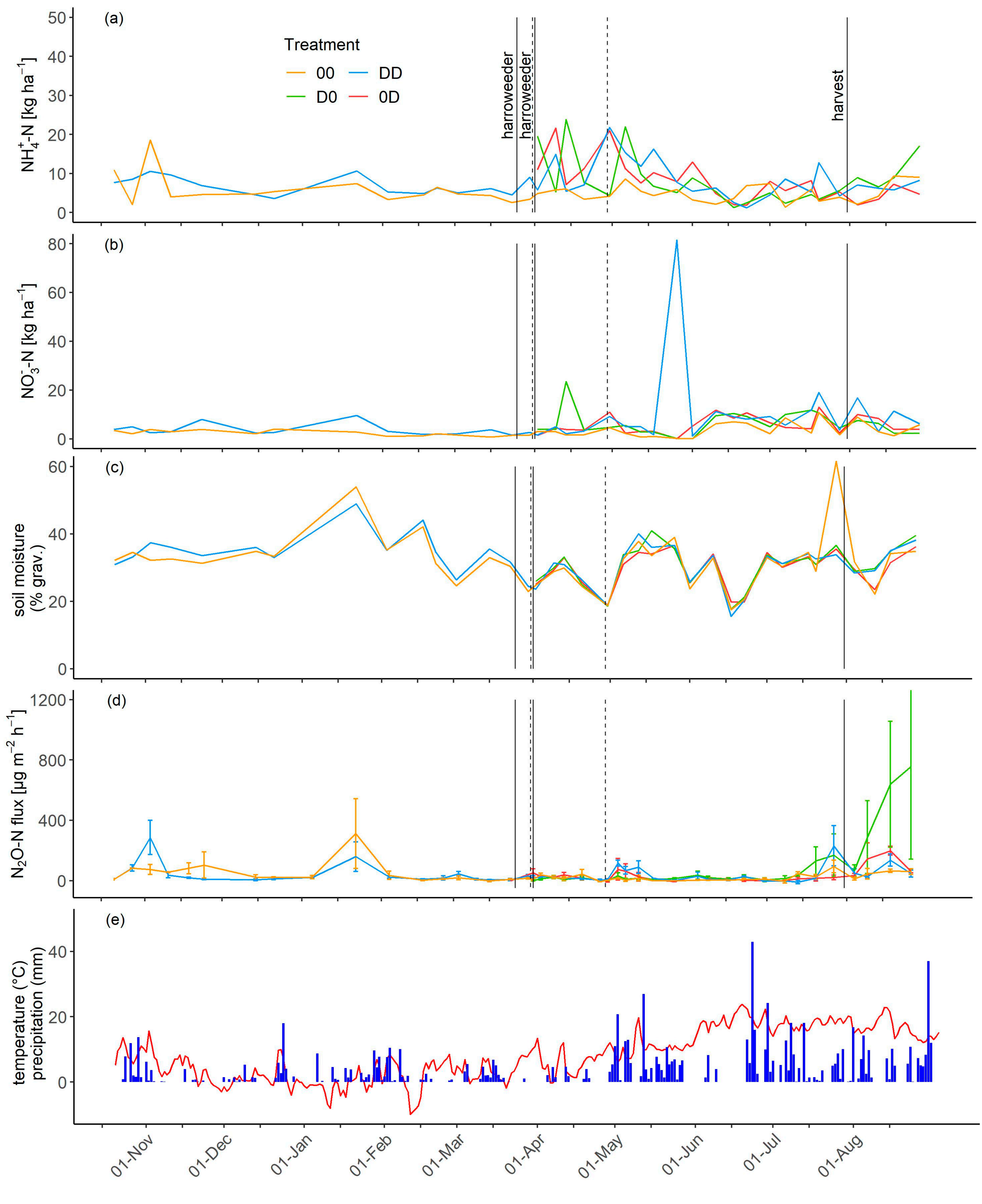

Figure 3. Ammonium-N (**a**) and nitrate-N dynamics (**b**) and soil moisture (**c**) of all four treatments related to the topsoil layer (0–15 cm) from October 2020 to September 2021. Nitrous oxide emissions (**d**), error bars illustrate the standard deviation) and temperature (red line) and precipitation (**e**) from October 2020 to September 2021. Black lines mark different agronomic actions and dashed lines mark the date of fertilization and only refer to the two fertilized treatments (DD and 0D). (Treatments: 00 long-term unfertilized, D0 first-time unfertilized, 0D first-time fertilized, and DD long-term fertilized).

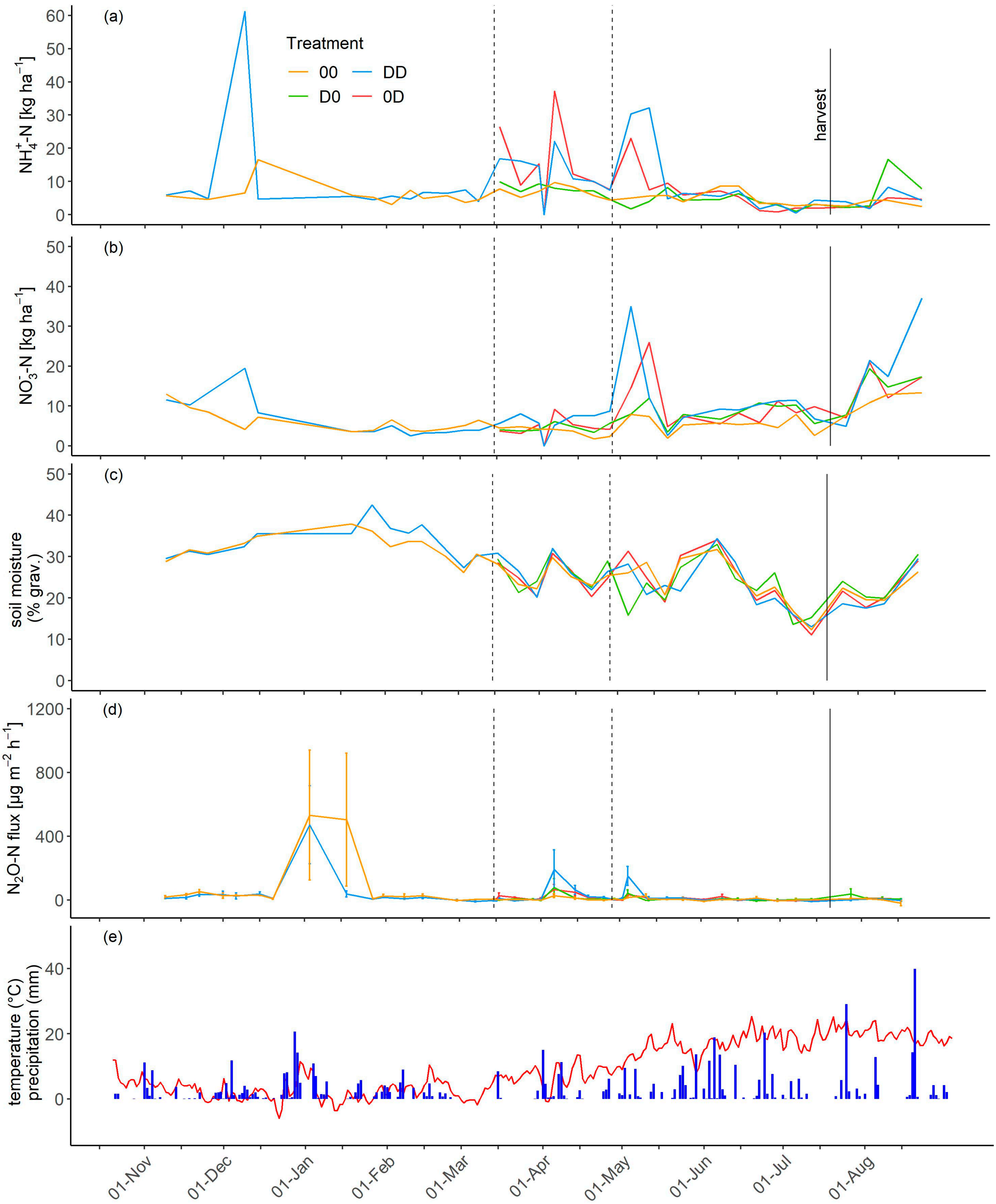

Figure 4. Ammonium-N (**a**) and nitrate-N dynamics (**b**) and soil moisture (**c**) of all four treatments related to the topsoil layer (0–15 cm) from October 2021 to September 2022. Nitrous oxide emissions (**d**), error bars illustrate the standard deviation) and temperature (red line) and precipitation (**e**) from October 2021 to September 2022. Black lines mark different agronomic actions and dashed lines mark the date of fertilization and only refer to the two fertilized treatments (DD and 0D). (Treatments: 00 long-term unfertilized, D0 first-time unfertilized, 0D first-time fertilized, and DD long-term fertilized).

Due to the N supply with digestate application, soil inorganic N stocks were temporarily higher in the fertilized treatments, which could have promoted nitrification and denitrification.

The increase in soil NH_4^+-N and NO_3^--N after the harvest can be attributed to tillage and the subsequent N mineralization of the harvest residues. An increase in soil NH_4^+ and NO_3^- stocks in spring was observed in all treatments, even in the unfertilized treatments. In addition to the N provided by the digestate, this increase is due to the soil N mineralization and the N transfer from the previous crop of clover–grass.

Soil moisture in % was very similar in all treatments throughout the period. In winter, the values leveled off at around 40 percent. The highest value was recorded on 10 February 2021 in 00 after a few rain events at the beginning of February with 43%. In spring and summer, there were greater fluctuations in water content due to plant growth and increased rainfall. The lowest value was measured on 14 July 2022 at 11% in the 0D treatment and the highest on 3 May 2019 in DD with 45% (Figures 1–4).

3.4. N_2O Fluxes

The unfertilized (00) treatment consistently showed lower N_2O-N emissions compared to the fertilized treatments. In 2019, N_2O-N fluxes were relatively low and unaffected by fertilization (Figure 1). In the 2019/2020 season (Figure 2), no significant N_2O-N emission peaks were observed in spring, and post-harvest emissions remained minimal. In 2020/2021 (Figure 3), N_2O-N emissions were very low throughout the growing season, with no notable increases following digestate application. However, in the winter of 2021/2022 (Figure 4), the 00 treatment exhibited N_2O-N emissions at levels comparable to the fertilized DD treatment, likely influenced by weather conditions such as rainfall and frost–thaw cycles.

The long-term fertilized (DD) treatment demonstrated notable N_2O-N dynamics throughout the study. In 2019, the DD treatment exhibited low N_2O-N fluxes in spring but emissions increased following heavy rainfall events and post-harvest tillage, temporarily exceeding 432 µg m^{-2} h^{-1} N_2O-N. In autumn 2019, a significant N_2O-N event was recorded in November. By spring 2020, there were pronounced emission peaks up to 1366 µg m^{-2} h^{-1} N_2O-N shortly after fertilizer application in the DD treatment. In winter 2021/2022, following rain and a subsequent temperature increase, N_2O-N emissions in the DD treatment reached levels comparable to those observed in the 00 treatment. Despite these variations, the DD treatment consistently emitted more N_2O-N due to increased soil NH_4^+ and NO_3^- stocks from fertilization.

The first-time fertilized (0D) treatment showed significant N_2O-N emission peaks. In 2019, fertilization led to slight and short-term increases in N_2O-N fluxes compared to unfertilized treatments. The 2019/2020 season showed pronounced emission peaks in spring, reaching up to 1366 µg m^{-2} h^{-1} N_2O-N after fertilizer application. Post-harvest in 2020, the highest N_2O-N fluxes were recorded in the 0D treatment, associated with elevated soil NO_3^- stock. In 2021, despite generally low emissions during the growing season, there was a slight increase in N_2O-N emissions following the second digestate application and subsequent precipitation.

The D0 treatment, without biogas digestate fertilization for the first time since 2005, also showed distinct N_2O-N emission patterns. In 2019, this treatment experienced a slight increase in N_2O-N fluxes following heavy rainfall events. In 2020, post-harvest emissions were higher in the D0 treatment, potentially due to increased N mineralization from previous fertilization.

3.5. Cumulative N_2O-N Emissions and Emission Factors

Table 6 presents the cumulative N_2O-N emissions in winter wheat, based on measured data as well as calculated with the GNOC model. Emissions are shown separately for winter and summer (from fertilization until harvest including post-harvest). Since strong post-harvest effects were evident in three of the four measurement years, the post-harvest emissions were calculated separately.

Table 6. Cumulated N_2O-N emissions (in kg ha^{-1}) of the investigated treatments and different phases of the vegetation of winter wheat in four years in comparison to the nitrous oxide emissions calculated with the GNOC model shown for the entire vegetation period from sowing to harvest. Different letters indicate significant differences (Tukey test, $p < 0.05$).

		Measured N_2O-N Emissions							Modelled N_2O-N Emissions	
Year of Harvest	**Treatment ***	**Autumn and Winter**	**Spring and Summer**	**Fertilization until Harvest**	**Post-Harvest**	**Whole Year**	**Product-Related**	**Emission Factor ****	**GNOC *****	**Emission Factor GNOC**
Unit	**kg ha^{-1}**	**kg ha^{-1}**	**kg ha^{-1}**	**kg ha^{-1}**	**kg ha^{-1}**	**kg ha^{-1}**	**kg Mg^{-1}**	**%**	**kg ha^{-1}**	**%**
2019	00		0.64 a	0.21 a	0.43 a				1.36	
	D0		0.96 a	0.23 ab	0.74 a				1.36	
	0D		1.06 b	0.53 d	0.53 a				3.28	0.82
	DD		1.49 c	0.75 c	0.74 a				3.28	0.82
2020	00	1.91	0.39 a	0.26 a	0.13 a	2.17	0.55 a		1.36	
	D0		1.16 a	0.38 a	0.78 a	2.50	0.57 a		1.36	
	0D		3.14 b	1.92 ab	1.22 b	3.83	0.56 a	0.63	3.70	0.88
	DD	2.12	3.30 b	2.50 b	0.79 b	4.62	0.66 a	0.93	3.70	0.88
2021	00	2.38	0.80 a	0.53 a	0.27 a	2.81	0.52 a		1.36	
	D0		3.34 b	0.78 a	2.56 b	2.51	0.42 a		1.36	
	0D		1.21 ab	0.48 a	0.73 ab	2.86	0.43 a	0.02	3.24	0.82
	DD	1.73	1.40 ab	0.74 a	0.66 ab	2.47	0.32 a	−0.15	3.24	0.82
2022	00	3.72	0.16 a	0.14 a	0.05 a	3.86	0.82 a		1.36	
	D0		0.32 ab	0.26 ab	0.06 a	2.30	0.35 a		1.36	
	0D		0.42 ab	0.38 bc	0.04 a	4.10	0.51 a	0.11	3.17	0.81
	DD	2.04	0.70 b	0.69 c	0.02 a	2.73	0.33 a	−0.51	3.17	0.81

* Treatments: 00 = long-term unfertilized, D0 = first-time unfertilized, 0D = first-time fertilized, and DD = long-term fertilized. ** The emission factor resulted from the cumulated emissions of the fertilized treatment ($Flux_{fertilized}$) minus the emissions of the control unit ($Flux_{control}$) in relation to the N input (Ninput). *** GNOC is based on an exponential algorithm that considers site- and management-specific characteristics such as soil texture, climate, soil organic matter, pH, and vegetation on the approach of Stehfest und Bouwman, 2006. For definition of the different vegetation periods check Table A2.

In 2019, summer emissions were 1.49 kg ha^{-1}. In 2020, they reached to 3.30 kg ha^{-1}, much higher than the unfertilized treatment (00) at 0.39 kg/ha. Post-harvest emissions in 2020 were 2.50 kg ha^{-1}, again higher than the unfertilized treatments (00: 0.26 kg ha^{-1} and D0: 0.38 kg ha^{-1}).

In 2021, winter emissions in the DD treatment were higher than in 00, but not significantly. During the winter of 2021/22, DD emissions were very high at 2.04 kg ha^{-1}. The 00 treatment usually had the lowest emissions. In 2019, these were 0.64 kg ha^{-1}. In 2020, winter emissions were slightly lower than DD but not significantly and summer emissions were 0.39 kg ha^{-1}. In winter 2021/22, very high emissions for 00 at 3.72 kg ha^{-1} were observed.

The 0D treatment had intermediate emissions. In 2019, they were higher than 00 but lower than DD. In 2020, emissions from fertilization to harvest (1.92 kg ha^{-1}) were similar to the long-term fertilized treatment. The D0 treatment emitted 0.38 kg ha^{-1} in 2020 from fertilization to harvest, less than DD. In 2021, D0 had high emissions due to a strong post-harvest effect, with the highest post-harvest emissions at 2.56 kg ha^{-1}. No substantial differences were observed from fertilization to harvest.

Product-related N_2O-N emissions refer to the whole year and are therefore presented for the years 2020–2022 (Table 6). Due to high wheat yields and relatively low emissions, the product-related N_2O-N emissions are at a very low level (0.35–0.82 kg Mg^{-1}), without significant differentiation between the fertilization treatments and years.

For the calculation with the GNOC model, only the current fertilization was taken into account, neither previous crops nor past fertilization. Thus, there is no difference in the N_2O-N emissions between the long-term and first-time treatments. The emissions of the fertilized treatments were 3.17 to 3.70 kg ha^{-1} N_2O-N, while those of the unfertilized treatments were 1.36 kg ha^{-1} N_2O-N. The emission factors could only be calculated for the fertilized treatments. The GNOC emission factor was between 0.82 and 0.88%. Emission factors based on measured emissions were also very low. As emissions from 00 exceeded DD in 2021 and 2022, negative rates were calculated.

4. Discussion

The implementation of a nitrous oxide measurement campaign in an already established long-term field experiment provided the opportunity to analyze the long-term impacts of biogas digestate that have accumulated over many years [36,57]. N_2O measurement results from long-term experiments with different fertilization are still rare, especially in organic farming [58,59]. As fermentation residues contain a high proportion of organically bound C and N (Table 4), it is to be expected that considerable amounts of C and N are stored in soil organic matter. In fact, SOC and TN stocks were significantly higher in the fertilized plots [39]. With the long-term SOC and TN accumulation, the C and N mineralization potential increases [60], so that even with discontinued fertilization (treatment D0), more N is mineralized from the soil, which can potentially cause N_2O emissions.

One characteristic of the trial is the clover/grass–winter wheat crop sequence, which is typical for organic farming in temperate climates [61]. In the trial, the clover–grass was cut and the aboveground biomass was removed; therefore, only harvest residues and roots remained in the soil. Due to the high clover–grass yields in the trial (up to 15 Mg ha^{-1} dry matter yr^{-1}) and the high biological N fixation rate (up to 450 kg ha^{-1}) [38,39,62], significant amounts of C and N remained in the residues. The mineralization patterns were different depending on the ploughing date and the weather conditions. The N transfer from clover–grass to winter wheat [63,64] is one explanation for the sometimes high N_2O-N fluxes in the unfertilized treatment in autumn/winter [65–67].

The measurement of N_2O-N emissions using the closed-chamber method is widespread and, as in this work, is most commonly used for the measurement of N_2O-N fluxes. However, the high temporal and spatial variability of nitrous oxide formation in soils represents a major methodological problem [68]. Lammirato et al. [69] state that the natural spatial variability of fluxes measured in repeated plots of the same treatment using the closed-chamber method is considerable, making it difficult to identify statistically significant differences in emissions between different fertilized treatments [69]. This problem becomes more apparent when N_2O fluxes are high, since the spatial variability of fluxes appears to be positively correlated with the magnitude of the fluxes [70]. It is also necessary to consider the temporal variability of N_2O fluxes. According to Reeves and Wang [49], the most efficient sampling schedule is weekly, with an increased sampling frequency after heavy rainfall events. Nevertheless, not all N_2O emission peaks can be reliably detected, which may influence the results.

Above all, the large number of factors and their interactions that influence the formation of nitrous oxide in arable soils represent a major challenge when modeling N_2O fluxes and deriving mitigation strategies [71]. Therefore, in addition to modeling N_2O fluxes, such as with the GNOC method, measurements in the field under practical conditions are still essential [72,73]. N_2O measurements from field experiments are also used to validate soil process models and to test N_2O emission factors [73].

Digestate fertilization (DD treatment) significantly increased crop yields, ranging from a 53% increase in 2019 to an 83% increase in 2022 compared to the unfertilized control (00 treatment). This substantial yield improvement is attributed to the high and repeated applications of digestate, which supplied up to 265 kg N ha^{-1} yr^{-1}, including 129 kg NH_4^+-N ha^{-1} yr^{-1} in 2020. These results are consistent with previous studies showing similar crop yield increases after digestate fertilization in long-term field experiments and conventional farms [22,23,29,30,74].

The treatment fertilized for the first time (0D) did not reach the yield level of the long-term fertilized treatment (DD). The yield difference was 0.5 to 1.2 Mg ha^{-1} or 6% to 15%, although it was only significant in 2019. Fouda et al. (2011) showed that biogas digestate fertilization results in increases in SOC and total N. This also assumes that the plant-available N in the digestate is at least equal to the proportion of NH_4^+-N and that the fertilizer effect is therefore very good even in the first year [75].

The experimental data demonstrate the after-effect of fertilization, which is related to soil N accumulation due to the organic N added with the digestate, that can lead to increased N mineralization in the long term [76]. The after-effect of long-term fertilization with biogas digestate has not yet been investigated under field conditions. Our results show higher yields of D0 treatment than 00 treatment and slightly higher N_2O-N emissions, although these were not always significant. As the fertilizer has been added for years, the SOC and SON accumulate and thus results in an increased N mineralization potential [77].

The relevant weather conditions for the yield formation of winter wheat were favorable in 2019 and 2022, but unfavorable with pronounced dry periods in April and May 2020, which is a critical period for the biomass development of wheat [78]. This is reflected not only in low yields in 2020, but also in a very high N surplus of up to 133 kg ha^{-1}, indicating a high N loss potential. The highest N_2O-N emissions in the vegetation period were also measured in this year. With the increase in temperature from the beginning of April, there is also an increase in nitrous oxide emissions in 2020. At this time, maximum nitrous oxide fluxes occur when dry soils are rewetted [79,80].

The increase in N_2O-N emissions post-harvest and subsequent tillage was observed in three trial years. Previous studies have also reported increasing N_2O emissions post-harvest due to higher SOC availability triggered by the incorporation of crop residues during soil tillage [81–83]. In some studies, N_2O emissions after the wheat harvest accounted for up to 50% of the total annual N_2O emissions [83]. Stubble tillage and precipitation often results in good conditions for the mineralization of crop residues and soil organic N, which can lead to an increase in soil NH_4^+ and NO_3^- stocks as well as N_2O emissions [84,85]. An increase in soil mineral N stocks after the wheat harvest was observed in all treatments (including the 00 treatment). Nevertheless, post-harvest N_2O-N emissions were the lowest in the control treatment 00 (significant differences in 2020 and 2021).

The N balance for the fertilized treatments showed high N surpluses in some years. The significantly higher TN contents of the long-term fertilized treatments (Table 1) suggests that a significant portion of N not taken up by plants was stored in the soil rather than emitted into the environment. Particularly, this is relevant for interpreting the N balance data, where unfertilized treatments tend to underestimate emissions and fertilized treatments may overestimate them, as seen in 2021 and 2022. However, in 2020, emissions were higher due to the high N_2O-N fluxes caused by digestate application, emphasizing the influence of site-specific and crop rotation factors on emission potential. Site-specific conditions such as soil properties and weather conditions are mutually dependent and influence N_2O emissions. These findings are corroborated by measurements in different maize treatments within the same experiment [86].

The GNOC model underestimated the N_2O emissions for the unfertilized treatments. The N_2O emissions of the fertilized treatments were overestimated in 2021 and 2022. In 2020, the measured N_2O emissions were higher than the modelled N_2O emissions. In 2022, high winter emissions were detected for the 00 treatment resulting in high N_2O-N emissions in 0D. This shows again that site and weather conditions as well as crop rotation and agronomic management have a high impact on emissions and can exceed fertilizer-induced emissions. This is also confirmed by measurements in the same experiment in different maize treatments [86].

For further investigations, it must be taken into account that biogas digestate contains organic C compounds that are recalcitrant, but also rapidly degradable compounds that contribute to humus and SOC accumulation [87,88]. Levin et al. (2021) and Simon (2021) found close relationships between digestate input and SOC and N stocks in the same field trial [38,39]. Since C and N cycles are closely linked [89], the SOC influences the N turnover in soil [90] and hence the production of nitrous oxide.

5. Conclusions

The study found that digestate fertilization significantly increased area-related N_2O emissions compared to unfertilized treatments, but did not increase product-related emissions. The results indicate that the integration of a biogas plant and the application of biogas digestate are suitable measures for organic farming, despite the N_2O losses from digestate fertilization. It was shown that N_2O emissions are not only the result of fertilization, but also can be caused by previous crops, tillage, and post-harvest management and vary significantly depending on the weather conditions and annual effects. Further investigations should consider C sequestration by the digestate and examine whether this can offset N_2O emissions.

The extensive data set can be used to calibrate and validate models for analyzing greenhouse gas fluxes. The authors intend to publish the data set and thus make it accessible to the scientific community.

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Appendix A

Table A1. Date of fertilization and N application rate.

Date	26 March 2019	26 April 2019	23 March 2020	23 April 2020	31 March 2021	29 April 2021	14 March 2022	28 April 2022
Treatment *	BBCH 20/21	BBCH 30/31	BBCH 20/21	BBCH 30/31	BBCH 20/21	BBCH 30/31	BBCH 20/21	BBCH 30/31
00	-	-	-	-	-	-	-	-
D0	-	-	-	-	-	-	-	-
0D [kg ha^{-1}]	116.7	116.7	132.3	132.3	114.4	114.4	111.6	111.6
DD [kg ha^{-1}]	116.7	116.7	132.3	132.3	114.4	114.4	111.6	111.6

* 00 long-term unfertilized, D0 first-time unfertilized, 0D first-time fertilized, and DD long-term fertilized.

Table A2. Different phases of the vegetation of winter wheat in four years with exact dates.

	Autumn and Winter	Spring and Summer	Fertilization until Harvest	Post-Harvest	Whole Year
2019		26 March 2019–03 September 2019 (161 days)	26 March 2019–24 July 2019 (120 days)	24 July 2019–03 September 2019 (41 days)	
2020	14 October 2019–24 March 2020 (162 days)	24 March 2020–25 August 2020 (154 days)	24 March 2020–28 July 2020 (126 days)	28 July 2020–25 August 2020 (28 days)	14 October 2019–28 July 2020 (288 days)

Table A2. *Cont.*

	Autumn and Winter	Spring and Summer	Fertilization until Harvest	Post-Harvest	Whole Year
2021	20 October 2020–30 March 2021 (161 days)	01 April 2021–26 August 2021 (147 days)	01 April 2021–27 July 2021 (117 days)	27 July 2021–26 August 2021 (30 days)	20 October 2020–27 July 2021 (280 days)
2022	09 November 2021–14 March 2022 (125 days)	14 March 2022–16 August 2022 (155 days)	14 March 2022–20 July 2022 (128 days)	21 July 2022–16 August 2022 (26 days)	09 November 2021–20 July 2022 (253 day)

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