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Effects of Organic Maize Cropping Systems on Nitrogen Balances and Nitrous Oxide Emissions

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Abstract: Silage maize cultivation is gaining importance in organic farming, and thus its environmental and climate impacts. The effects of digestate fertilization in combination with different catch crops and tillage intensities in maize cultivation are investigated in a long-term field experiment in southern Germany. The tested variants are (a) maize after winter rye, plowed, unfertilized and (b) fertilized with biogas digestate, (c) maize after legume-rich cover crop mixture, mulch seeding, fertilized with digestate, and (d) maize in a white clover living mulch system, fertilized with digestate. Over three years (2019 to 2021), crop yields and N balance were analyzed, N_2O emissions were measured in high temporal resolution using the closed chamber method, and soil moisture, ammonium, and nitrate contents were continuously determined. Maize dry matter yields ranged from 4.2 Mg ha^{-1} (variant a, 2021) to 24.4 Mg ha^{-1} (variant c, 2020) depending on cropping intensity and annual weather conditions. Despite relatively high nitrogen fertilization with digestate, the N balances were negative or nearly balanced; only in 2021 did the N surplus exceed 100 kg ha^{-1} (variant b and c) due to low yields. In maize cultivation, relatively low N_2O-N emissions (1.0 to 3.2 kg ha^{-1}) were measured in the unfertilized variant (a), and very high emissions in variant b (5.6 to 19.0 kg ha^{-1}). The sometimes extremely high N_2O emissions are also due to soil and climatic conditions (high denitrification potential). The experimental results show that cover crops, living mulch, and reduced tillage intensity in silage maize cultivation can reduce N_2O emissions, improve nitrogen balance and increase maize yields.

Keywords: nitrous oxide; soil nitrogen dynamics; biogas digestate; tillage operation; cropping system; cover crops; greenhouse gas; organic farming; energy crops

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1. Introduction

1.1. Social and Scientific Relevance

Presently, about 9% of the EU's agricultural area is farmed organically [1], and the trends show that with the present growth rate, the EU will reach 15–18% by 2030. One overall aim of the European Green Deal is to boost the production and consumption of organic products, to reach 25% of agricultural land under organic farming by 2030 (European Commission, 2021).

This also brings the environmental and climate impacts of organic farming into sharper focus, including the question of whether increasing organic farming acreage leads to a reduction in greenhouse gas (GHG) emissions. Of particular importance here are nitrous oxide (N_2O) emissions. N_2O is a long-lived GHG with an atmospheric concentration of currently 331 ppb [2–4] (from 1750 to 2017, the concentration increased by 22% from 270 ppb to 331 ppb [5]). Currently, the increase per decade is 2%. The atmospheric residence time of N_2O is 114 years and its CO_2 equivalent is 298 relevant in 100 years [2]. N_2O is formed during the microbial conversion of nitrogen by nitrification and denitrification in the soil [6,7]. The main cause of the increase in N_2O concentration is agriculture, which accounts for up to 80% [8].

Numerous interacting factors influence N_2O emissions in crop production systems. The first to be mentioned are the N fertilizers [9–11]. Moreover, the cultivation system [12], tillage [13,14], crop rotation [13,15], catch crops [16,17] and timing of agronomic operations [18] have an impact on the formation of N_2O in agricultural used soils. Due to this complexity and the numerous interactions, it is so difficult to assess the N_2O loss potential of cropping systems. This is also shown by the partly contradictory results on N_2O emissions in organic farming systems [19]. The mechanisms, influencing factors and interactions need to be known in order to derive site-specific management recommendations to mitigate N_2O fluxes [20]. Since the anthropogenic factors are modifiable they offer the possibility of achieving a reduction in GHG-emissions through adapted management [21]. As the reduction in greenhouse gases is imperative, possible mitigation strategies must be implemented in agriculture [22,23].

1.2. Need for Research and Development

Compared to conventional agriculture, studies in organic agriculture show lower fossil energy input and associated CO_2 emissions [24–26], higher soil carbon sequestration [27], and predominantly lower N_2O emissions per hectare of cropland [28]. Numerous studies are already available on N_2O emissions in organic farming [19,29,30]. Here, partly contradictory results were found–also due to the diversity of cultivation systems. Some specifics of organic farming (no use of mineral fertilizer nitrogen, limited livestock, diverse crop rotations with intercropping) lead to comparatively low or moderate area-related N_2O emissions [28]. On the other hand, high N_2O emissions can occur in organic farming, including clover-grass mulch systems with intensive green manuring [31], after long-term organic fertilization [32,33], or cultivation of legumes [16]. N_2O emissions from new organic farming systems have been insufficiently studied. These include energy crop rotations with maize and digestate fertilization, which have gained importance due to the massive expansion of biogas production [34]. In general, silage maize cultivation has increased significantly in organic farming due to high energy yields, efficient cultivation practices, high digestibility and methane formation potentials of maize [35,36]. The use of renewable raw materials for the production of sustainable energy should primarily conserve fossil raw materials and contribute to the reduction of man-made greenhouse gases, thus helping to combat climate change [37]. This only works if the emissions from the production of energy crops and fertilization with biogas digestates, the nutrient-rich residue of biogas production, are lower than those produced by fossil fuels. Biogas digestate has the potential to function as a fertilizer or soil amendment and nitrogen source for agricultural production [38]. The influence of digestates on soil quality and fertility [39], the biological properties [40], soil chemical properties, and crops yields have been studied [41]. Current studies show that the application of biogas digestate can achieve comparable [42] or even higher yields [43] than with unfermented organic fertilizers. The expansion of the sector will inevitably lead to an even greater amount of digestate, which, due to its high amounts of nitrogen, inevitably causes nitrous oxide emissions. However, these are lower than in the case of fertilization with urine or manure [38].

Studies on tillage show controversial results regarding N_2O emissions. Few found lower emissions with reduced tillage [14] or even higher emissions [44,45]; and some found no difference [20]. Reduced tillage can lead to increased water and lower oxygen content, which in turn promotes denitrification, whereas it might be neutral in well-drained soils [46]. In addition, the effect of reduced tillage on soil organic carbon (SOC) must be considered. Reduced tillage may lead to higher nitrous oxide fluxes, but these could be offset by an accumulation of organic carbon in the soil [47,48]. This effect could be intensified by fertilizing with digestate due to its high amount of stable C compounds [49–51]. The use of the plow generally leads to a mineralization boost, which can lead to increased N losses in form of N_2O [52,53].

Moreover, the use of winter cover crops or an already established living mulch-seed can be advantageous [54–56]. These affect the organic C and N content of the soil, water balance, soil temperature and sowing time of maize and must be taken into account [57]. The fungal and microbial activity under living mulch is amplified and could lead to an increase in N_2O [54]. Due to the N fixing capacity of legumes less fertilizer needs to be applied, which can have a positive, or neutral, effect on nitrous oxide emissions [58]. In addition, the living mulch always competes with the main crop and can have a negative effect on yield [55].

When evaluating N_2O and GHG fluxes, it should be noted that yields are significantly lower in organic farming [59]. Thus, low area-related GHG and N_2O emissions do not always correspond to low product-related GHG emissions [28,60]. Therefore, the assessment of GHG emissions should be linked to an agronomic assessment, especially the yield performance of the cropping system [61]. Experimental data about N_2O fluxes in maize cultivation under organic conditions are limited [33].

1.3. Purpose and Objectives

In the present work, N_2O fluxes in different silage maize cultivation systems under organic farming conditions are analyzed in a long-term field experiment with high temporal resolution using the closed-chamber method. The experiment is located in southern Germany (40 km north of Munich) on a site with high yield potential. The four maize cultivation systems differ in terms of catch crop preceding maize (freezing legume mixture, frost resistant perennial rye, white clover living mulch), tillage intensity (plow vs. mulch), and fertilization (with/without digestate), in an otherwise equal crop rotation (consisting of winter wheat, clover grass, triticale, and maize). These cultivation systems represent different maize cultivation and include, in addition to the systems commonly used in practice, a particularly soil-conserving and erosion-reducing variant (maize in the white clover living mulch system).

To interpret the N_2O fluxes, soil moisture dynamics, as well as soil nitrate and ammonium dynamics, are investigated. In addition to area-based cumulative N_2O emissions and product-based N_2O emissions are calculated. On the basis of yield, protein content, nitrogen removal, and nitrogen balance, an agronomic evaluation of the cultivation systems is carried out.

The aim of the experiment is to clarify whether environmentally relevant N_2O fluxes occur in organic maize cultivation, especially after intensive fertilization and tillage in spring (in a phase with potentially high N mineralization) and whether N_2O emissions can be reduced significantly by cultivation measures.

Hypotheses 1. *Fertilization with digestate causes a significant increase in N_2O fluxes in organic maize cultivation.*

Hypotheses 2. *Conventional tillage (plowing) prior to maize sowing stimulates soil N mineralization and increases soil nitrate levels as well as N_2O emissions.*

Hypotheses 3. *Conservation tillage decreases N mineralization and N_2O fluxes (compared to plow tillage) without yield reduction.*

Hypotheses 4. *Maize cultivation in living mulch systems results in low N_2O emissions, but also in significant yield losses.*

2. Materials and Methods

2.1. Study Site and Weather Conditions

The field trial was conducted in the tertiary hill country, a hilly landscape consisting of unconsolidated Tertiary sediments overlain by a thin loess cover in the research station Viehhausen (30 km north of Munich and 490 m above sea level (48°39′62′54 N, 11°65′07′31 E). The soil is a Haplic Luvisol [62] with 25% clay, 62% silt and 13% sand (silty loam). Climatic conditions are humid and cool and soils are, due to high clay content, heavy, warm up slowly and tend to condensate and clog.

The long-term average temperature and precipitation for the site are 8.1 °C and 792.9 mm per year. The years 2019 and 2020 were characterized by a dry and warm spring, especially in April, followed by periods of heavy rainfalls in summer. The year 2021 was characterized by a rather cool spring. April and May were even cooler than the long-term average.

Moreover, the winter of 2019/20 was very mild compared to the long-term average with hardly any frost days. Compared to the long-term average, the three years covered by the study are all warmer on average. The amount of precipitation in 2019 and 2020 is similar to the long-term average, in 2021 even 121.5 mm more (Table 1).

Table 1. Monthly temperature and precipitation for the years 2019–2021 and the long-term average (1981–2010), experimental station Weihenstephan-Dürnast.

	Unit	January	February	March	April	May	June	July	August	September	October	November	December	Mean
1981–2010														
Temperature	[°C]	−1.5	−5	3.9	7.8	12.8	15.4	17.4	17.2	12.9	8.4	3.1	−1.0	8.1
Precipitation	[mm]	44.1	35.9	50.4	48.6	82.8	88.4	10.6.0	87.5	73.3	58.5	55.3	59.8	792.9
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
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

2.2. Experimental Set Up

The investigations were carried out in the long-term field experiment "energy crop rotation". The field experiment was set up in 2005 and covers an area of 3.75 hectare. It consists of variants with different crop rotations, different fertilization and tillage systems with four replications (384 plots).

The investigations were conducted in 4 trial variants (see Table 2) with 4 replications (N_2O measurement with 3 replications) over 3 years. The crop rotation consists of triticale−clover grass−winter wheat−cover crops−maize (see Appendix A Figure A1). Crop rotations differ only with regard to intercropping before maize. Each plot made up a total of 72 m^2 (12 m × 6 m) [63,64].

Table 2. Characterization of the test variants.

Crop Sequence [a]	**Tillage System [b]**	**Biogas Digestate [c] [m^3]**	**N Input 2019 [kg ha^{-1}]**	**N Input 2020 [kg ha^{-1}]**	**N Input 2021 [kg ha^{-1}]**
Winter rye—maize	CT	0	0	0	0
Winter rye—maize	CT	40	233	264	229
Catch crop mixture—maize	RT	40	233	264	229
White clover—maize	LM	30	175	198	172

[a] Combination of winter catch crop and following main crop maize. [b] CT = conventional tillage (plowing), RT = reduced tillage (mulching), LM = living mulch. [c] System-compliant application of digestate.

2.3. Biogas Digestate and Agronomic Management

The biogas digestate was produced by a local organic farmer from a feedstock mixture corresponding to the biomass produced in the trial (clover grass, maize silage). Digestate was applied using a slurry tanker fitted with trailing hoses. The chemical composition of the digestate is summarized in Table 3. After application, the digestate was incorporated into the soil (except in the living mulch variant).

Table 3. Chemical composition of the biogas digestate applied to maize in 2019 and 2021.

Parameter	Unit	2019	2020	2021
Dry matter (DM)	%	9.55	9.20	8.3
Tot-C	% DM	38.85	38.70	42.03
Tot-N	% DM	6.11	7.17	6.89
NH_4-N	% Tot-N	41.73	48.68	52.25
C:N		6.36	5.38	6.1
K_2O-K	% DM	8.02	8.82	9.93
Tot-S	% DM	0.51	0.49	0.55

The production processes (field operations) are presented in detail in Appendix A. All management operations were adapted to the respective variant with consideration of catch crops, weather and soil conditions, weed pressure, and plant stands.

2.4. Biomass, Soil Samples and Laboratory Analyses

For the determination of the biomass yield a manual plant cut was made at harvest time (2 m^2). Then, the crop biomass was dried at 105 °C. Dry matter was ascertained and extrapolated to one hectare. The determination of the N and C content of the biomass was carried out with an Elementar Vario MAX C/N analyzer [65]. For the analysis of mineral nitrogen (ammonium and nitrate N) content, weekly soil samples were taken with an auger at depths of 0–15 cm and 15–30 cm. One soil sample consisted of one composite sample of the four replicates. The soil samples were homogenized and then solved in 1.0 M KCl extract to analyze nitrate, and ammonium content [66]. The gravimetric water content was determined by drying at 105 °C in a compartment drier.

N uptake is calculated from the dry matter yield and biomass N content. The N surplus is defined as the difference between the N input by the fertilizer (digestate) and the N output (N removal) in the maize biomass yield.

2.5. N_2O Measurement

The gas samples were obtained using the manual closed-chamber method [67] after the guidelines of the Nitrous Oxide Chamber Methodology [68]. To ensure the tightness of the gas measurement system, a frame was fixed into the soil. The chamber, equipped with a rubber seal, a fan, and a degassing hose, was then put onto the frame. N_2O samples were obtained once a week and additionally event-related after fertilization, heavy rainfalls, tillage, or frost—thaw—cycles. In order to obtain representative N_2O measurement data for the whole day, the time of day between 08:30 and 11:00 was chosen for gas sampling. This should ensure that the mean temperature of a day is covered approximately to allow cumulation of the measured gas flows [69]. The chambers covered a surface area of 0.36 m^2 and enclosed the plants until they reached a height of one meter. From then on split chambers (Olfs et al. 2018) were used. The split chambers consist of two equally sized parts with a size of 78 cm and 51 cm in height. They enclose the plants with a foam seal in the middle, allowing gas measurements in high plant stands. The increase in gas concentration in the chamber allowed the N_2O fluxes to be detected. Therefore, every 20 min within one hour samples were taken into glass vials with a battery-operated sampler. The vials, sealed with a septum, were then analyzed using a gas chromatograph 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 program RStudio and the package "gasfluxes" [70]. The package offers different models for measured concentration-time relationships from static chambers within the function. In this study the fluxes were calculated for each chamber based on the slope of the gas concentration in the chambers over time (1 hour, 4 measurements) using the nonlinear 'HMR' model (developed by Hutchinson and Mosier (1981) and revised by Pedersen et al. (2010) [71]), and the robust linear model [72]. Considering the specific chamber temperature and volume, the nitrous oxide concentrations were converted into the unit µg m^{-2} h^{-1}, which was continuously used in the following stages of work.

2.6. Calculation of Cumulative N_2O Emissions

Cumulative N_2O emissions were calculated by linear interpolation between two measuring times [73–75]. In order to analyze the influences of seasons and vegetation periods, the cumulated emissions were calculated for several time periods (whole year, winter and summer). To perform a statistical comparison of the cumulative fluxes, the data were logarithmized and then analyzed using a linear mixed model. For this, the data were manipulated to obtain negative values out (N_2O-N + 50 µg m^{-2} h^{-1}) [73].

Product-related emissions refer only to the maize yield and the time from maize seed to harvest. Product-related emissions were calculated by N_2O emissions per hectare divided by maize dry matter yield per hectare [76].

2.7. Statistical Analysis

The statistics program RStudio version 4.0.3 (10 October 2020)) was used for the statistical evaluation of the data. The analysis of variance was carried o with a linear mixed effects model using the lmerTest Package [77] at a significance level of α = 0.05, whereas treatment and year were fixed factors and blocks and replicates random factors. Furthermore, all test factors were evaluated with the post-hoc test "Tukey" to detect significant differences between the variants using the Kenward–Roger approximation for the degrees of freedom using the Emmeans package [78] (significance level of α = 0.05). Significant differences were represented by small letters.

3. Results

3.1. Effect of Biogas Digestate Fertilization on Biomass Yield and N Balance

Maize dry matter yields in the long-term unfertilized control variant reached 13.8 Mg ha^{-1} in 2019, 10.6 Mg ha^{-1} in 2020, and 4.2 Mg h^{-1} in 2021 (Table 4) and therefore reached the significantly lowest yields in all three test years. The different fertilized treatments showed significantly different yields in all three years.

Table 4. Yield, N content, N uptake, N surplus. Different letters indicate significant differences (Tukey test, $p \leq 0.05$).

Year	Treatment	DM Yield Mg ha^{-1}	N Input kg ha^{-1}	N Content %	N Uptake kg ha^{-1} Maize	N Surplus kg ha^{-1} Maize	N Uptake kg ha^{-1} Maize and Rye	N Surplus kg ha^{-1} Maize and Rye
2019	Winter rye—maize, CT, unfertilized	13.6 a	0	0.9 a	118.6 a	−118.6 a	227.2	−227.2 a
	Winter rye—maize, CT	21.1 b	233.0	0.8 a	176.0 ab	57.0 b	327.0	−94.0 b
	Catch crop mixture—maize, RT	19.3 b	233.0	1.0 a	193.5 b	40.0 b		40.0 c
	White clover—maize, LM	13.8 a	175.0	0.9 a	127.8 a	47.3 b		47.3 c
2020	Winter rye—maize, CT, unfertilized	10.5 a	0	1.0 a	96.9 a	−96.9 a	191.5	−191.5 a
	Winter rye—maize, CT	18.3 b	264.0	1.1 ab	199.7 b	64.3 c	312.0	−48.0 b
	Catch crop mixture—maize, RT	24.4 c	264.0	1.2 b	284.3 c	−20.3 b		−20.3 b
	White clover—maize, LM	17.8 b	198.0	1.1 ab	189.2 b	8.8 bc		8.8 b
2021	Winter rye—maize, CT, unfertilized	4.2 a	0	1.1 a	45.7 a	−45.7 a	73.5	−73.5 a
	Winter rye—maize, CT	6.5 ab	229.0	1.1 a	71.3 ab	155.5 c	135.0	94.0 bc
	Catch crop mixture—maize, RT	11.0 c	229.0	1.1 a	121.7 b	107.3 bc		107.3 b
	White clover—maize, LM	9.1 bc	172.0	1.2 a	107.2 b	64.8 b		64.8 b

In the first two study years, the fertilized winter rye—maize (plow) variant produced very high dry matter yields of 21.2 Mg ha^{-1} and 18.3 Mg ha^{-1}. However, the yields in 2020 were significantly exceeded by the catch crop mixture—maize (mulching) variant with 24.4 Mg ha^{-1}.

The white clover—maize (living mulch) variant did not yield significantly higher than the unfertilized winter rye—maize (plow) variant in 2019; however, it nearly reached the yield of the fertilized winter rye—maize (plow) variant in 2020. In 2021 it reached 9.1 Mg h^{-1} and thus performed well compared to the other variants in 2021. Overall, the yield level in the trial was very high in 2019 and 2020; in 2021, yields were comparatively low.

The N content of the silage maize ranged between 0.8 and 1.2% and was lowest in 2019. N uptake in maize yield was calculated based on dry matter yields and N contents. Extremely high N uptakes were found in the catch crop mixture—maize (mulching) variant (2020) with a maximum of 284 kg ha^{-1}. Even in the unfertilized variant, N uptakes of 96.9 (2019) and 118.6 kg ha^{-1} (2020), were recorded. The N uptake reflects the yields of the years and variants well and shows similar significant differences.

In the simplified N balance only, the direct N inputs by the digestate are considered, but not the N inputs by atmospheric N deposition, the soil N mineralization, and N transfer in the crop rotation (symbiotic N fixation of the clover grass). For the N surplus, only the unfertilized variants in all years and catch crop mixture—maize, RT in 2020 show negative values. The other variants are characterized by high N surpluses (highest surplus with 155.5 kg ha^{-1} in winter rye—maize, CT in 2021 because very high nitrogen quantities were applied. However, for winter rye—maize, CT variant, the N removal by winter rye must be included, which is why a second N surplus was calculated. This shifts the positive balance to a negative one (excluded from the year 2021).

In order to be able to make a statement about the N uptake of the different catch crops, a biomass cut of the catch crop stands was carried out before the first frost in October (see Appendix A Tables A4 and A5) and the N uptake of the biomass was determined for the catch crop mixture—maize, RT and the white clover—maize, LM variant. The winter rye was harvested in all three years at the beginning of May and N content was analyzed. The highest biomass in terms of dry matter was achieved in winter rye in 2019 and 2020, and in 2021 winter rye fertilized and leguminous catch crop reached equally high yields. In terms of N content, the catch crops variety and white clover performed significantly better than winter rye. Due to the high N contents and relatively high biomass values, the catch crop mixture—maize, RT achieved the significantly highest N uptake in all three years (Table 5).

Table 5. Dry matter yield and dry matter biomass cut, N content, and N uptake of the different catch crops. Different letters indicate significant differences (Tukey test, $p \leq 0.05$).

Year	Treatment [a]	DM Yield Mg ha^{-1}	DM Biomass Cut Mg ha^{-1}	N Content %	N Uptake kg ha^{-1}
2019	Winter rye—maize, CT, unfertilized	7.3 bc		1.5 a	108.6 ab
	Winter rye—maize, CT	8.9 c		1.71 ab	151 b
	Catch crop mixture—maize, RT		6.2 b	2.58 c	156.3 b
	White clover—maize, LM		3.5 a	2.22 bc	77.4 a
2020	Winter rye—maize, CT, unfertilized	8.4 c		1.18 a	94.6 a
	Winter rye—maize, CT	9.1 c		1.23 a	112.3 a
	Catch crop mixture—maize, RT		6.2 b	2.95 b	184.2 b
	White clover—maize, LM		4.2 a	2.57 b	109.5 a
2021	Winter rye—maize, CT, unfertilized	2.2 a		1.27 a	27.8 a
	Winter rye—maize, CT	4.8 b		1.32 a	63.6 a
	Catch crop mixture—maize, RT		4.5 b	2.96 b	133.7 b
	White clover—maize, LM		2.1 a	3.03 b	63.3 a

[a] CT = conventional tillage (plowing), RT = reduced tillage (mulching), LM = living mulch.

3.2. Ammonium and Nitrate Dynamics

In the following, two experimental variants (a) winter rye—maize, CT (conventional tillage) unfertilized and fertilized and (b) catch crop mixture—maize, RT (reduced tillage) and white clover—maize, LM (living mulch) are always shown together in one figure. Soil ammonium and nitrate dynamics are presented in line graphs for the time from April 2019 until October 2021 (Figures 1–3, note different axis scaling due to different values between the years and within the year in 2020).

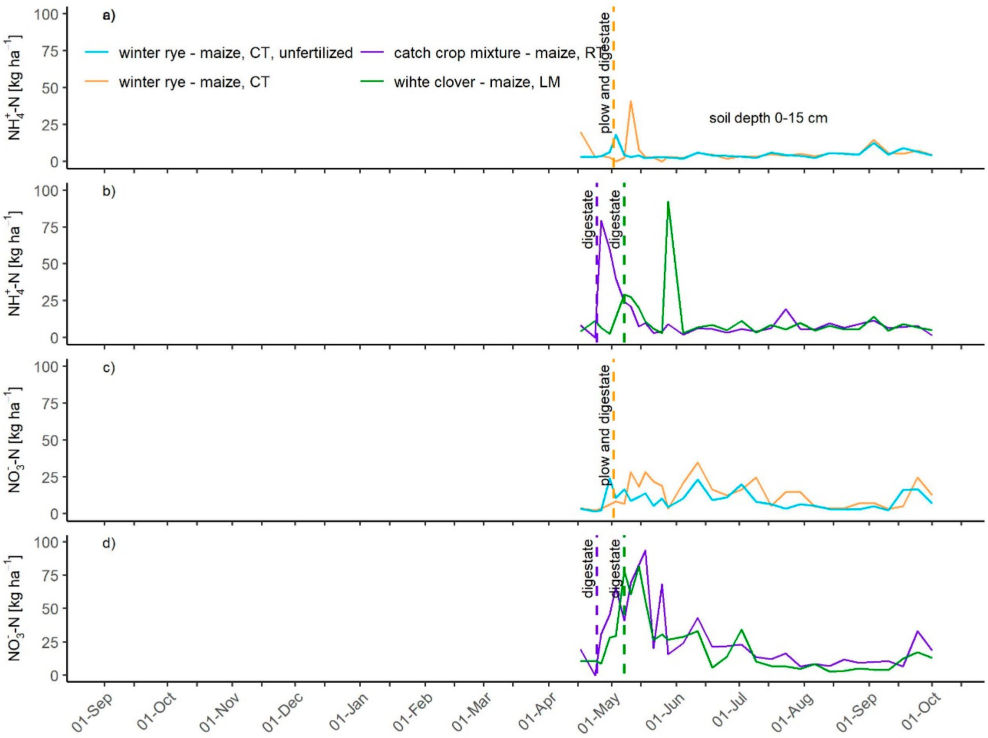

Figure 1. Ammonium and nitrate dynamics of all four variants related to the top-soil layer of 0–15 cm from April 2019 to September 2019. (**a**) Ammonium values in both winter rye—maize variants; (**b**) ammonium values in catch crop mixture—maize with reduced tillage and white clover—maize with living mulch; (**c**) nitrate values in both winter rye—maize variants; and (**d**) nitrate values of crop mixture—maize with reduced tillage and white clover—maize. Dashed lines indicate date of tillage adapted to each cultivation system, seed, and fertilization with biogas digestate.

An increase in soil ammonium and nitrate stocks was detected from late April/early May until July. The different fertilization, tillage, and sowing dates of the variants must be taken into account (see Appendix A Tables A2–A5).

In 2019, the highest NH_4-N stocks (92.2 kg ha^{-1}) were measured in the white clover—maize living mulch system on 28 May 2019 and the highest NO_3-N stocks (93.7 kg ha^{-1}) in the variant catch crop mixture—maize on the 17.05.2019. Ammonium and nitrate stocks in the winter rye—maize variants were comparatively low. In 2020 highest NH_4-N stocks (231.5 kg ha^{-1}, 30 April 2020) were measured in the mulch maize. Nitrate-N stocks reached 178.9 kg ha^{-1} in the catch crop mixture—maize variant. In 2021, soil mineral nitrogen values were low compared to 2019 and 2020 and reached their maximum on 19 April 2021 with 40.1 NH_4-N kg ha^{-1} (white clover—maize, LM) and on 12 May 2021 in the catch crop—maize, RT (63.3 kg ha^{-1}). In 2021, the values in the plowed rye—maize variant were mainly observed in the lower soil layer (15–30 cm, Appendix B Figure A5).

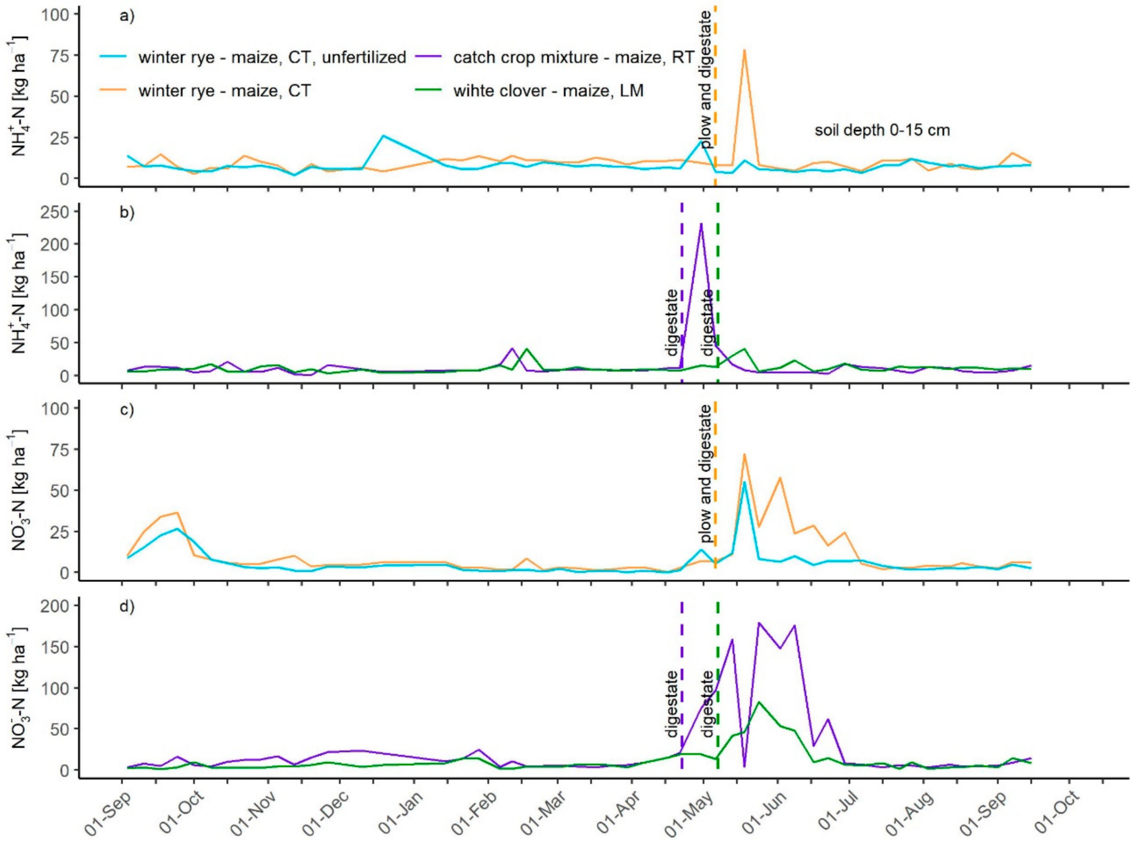

Figure 2. Ammonium and nitrate dynamics of all four variants related to the top-soil layer of 0–15 cm from September 2019 to September 2020. (**a**) Ammonium values in both winter rye—maize variants; (**b**) ammonium values in catch crop mixture—maize with reduced tillage and white clover—maize with living mulch; (**c**) nitrate values in both winter rye—maize variants; and (**d**) nitrate values of crop mixture—maize with reduced tillage and white clover—maize. Dashed lines indicate date of tillage adapted to each cultivation system, seed, and fertilization with biogas digestate.

Ammonium and nitrate stocks were low throughout both winters (Figures 2 and 3). After tillage and seeding of rye in September, there was a slight increase in ammonium values in the winter rye—maize variants. There were several frost–thaw events in early February followed by warmer temperatures that may explain the increase in nitrate stocks in the catch crop mixture—corn and white clover—maize variants.

It is noticeable that in all test years the ammonium and nitrate values decreased from July onwards and remained at a low level. The reduction in soil mineral nitrogen stocks occurred simultaneously with the increasing biomass formation and nitrogen uptake of the maize.

3.3. Soil Moisture

Although the variants have experienced different intercrops and tillage, there are hardly any differences in the water contents. Depending on the annual weather and stand development (evapotranspiration), relatively high soil water contents were found after intensive precipitation events (e.g., in spring), and low soil water contents in some periods in summer (see Appendix B Figure A6).

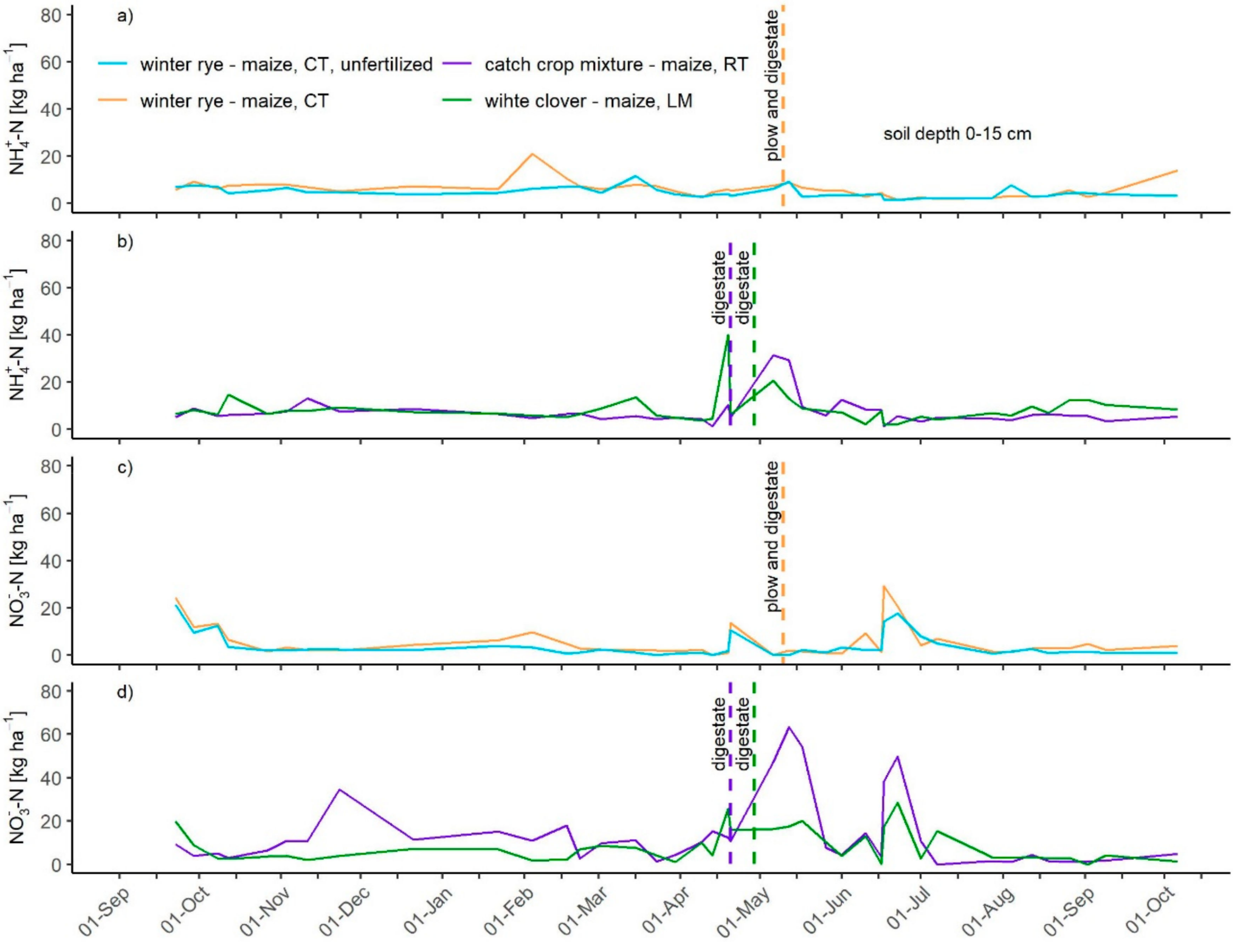

Figure 3. Ammonium and nitrate dynamics of all four variants related to the top-soil layer of 0–15 cm from September 2020 to September 2021. (**a**) Ammonium values in both winter rye—maize variants (**b**) ammonium values in catch crop mixture—maize with reduced tillage and white clover—maize with living mulch, (**c**) nitrate values in both winter rye—maize variants and (**d**) nitrate values of crop mixture—maize with reduced tillage and white clover—maize. Dashed lines indicate date of tillage adapted to each cultivation system, seed, and fertilization with biogas digestate.

3.4. N_2O Fluxes

Nitrous oxide emission peaks occurred in all three years of the trial. The bulk of the emissions occurred between the beginning of May and the end of July, coinciding with the increase in soil mineral nitrogen levels.

The nitrous oxide fluxes exhibit peaks in the period from May to July 2019. Time-delayed after biogas digestate fertilization, tillage, and seedbed preparation there is a peak in the fertilized winter rye—maize variant which lasted over a longer period and reached its peak on the 28 May 2019 with 1153.3 μg m^2 h^{-1} N_2O-N. The large scatter in Figure 4 shows the spatial variance of the measurements. N_2O peaks were observed in June and July 2019, but with high variability in N_2O fluxes. Figure 4a shows higher N_2O fluxes in the fertilized versus unfertilized winter rye—maize variant. The white clover—maize—living mulch variant (1172.8 μg m^2 h^{-1} N_2O-N on 11 June 2019) resulted in higher N_2O peaks than the catch crop mixture—maize variant (Figure 4b). The N_2O peaks occurred slightly earlier in the winter rye—maize variant than in the white clover—maize—living mulch variant.

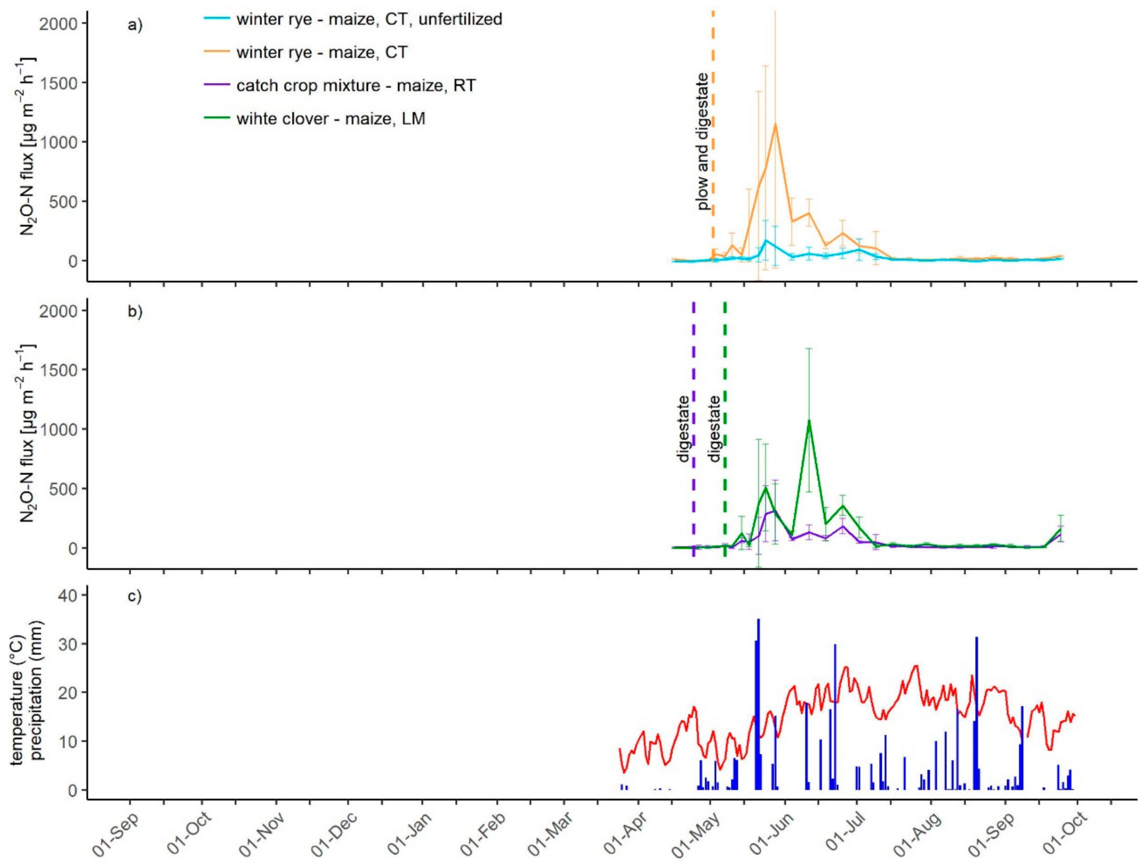

Figure 4. N_2O emissions for the treatments (**a**) winter rye—maize, CT, unfertilized and winter rye—maize, CT and (**b**) catch crop mixture—maize, RT, and white clover—maize LM from April 2019 until October 2019. Error bars illustrate the standard deviation. (**c**) Shows daily mean temperature and precipitation.

N_2O emissions were at a low level during the winter of 2019/20 (Figure 5).

N_2O emissions were higher in 2020 (Figure 5) than in 2019 (Figure 4) during some periods in spring/early summer. In the fertilized winter rye—maize variant, the N_2O fluxes were at a high level for a longer period until mid-July 2020, reaching their maximum with 2527.1 µg m^2 h^{-1} N_2O-N on 7 July 2020 (Figure 5a). There was a strong N_2O emissions event in the white clover—maize—living mulch variant in early summer 2020 (3036.0 µg m^2 h^{-1} N_2O-N on 12 June 2020).

In the winter of 2020/21, significant nitrous oxide fluxes were measured compared to the previous year (Figure 6). These occurred in all variants in October after heavy rainfall events and were the highest in the fertilized winter rye—maize variant (1269.7 µg m^2 h^{-1} N_2O-N), followed by the unfertilized control variant (1127.2 µg m^2 h^{-1} N_2O-N) on the 13 October 2020 (Figure 6a). Between December and the end of April, emissions stayed at a low level and increased after fertilization and tillage.

In winter rye—maize, however, these are lower than in previous years (1437.8 µg m^2 h^{-1} N_2O-N on 22 June 2021). Noticeable is the very high peak in the catch crop—maize variant with reduced tillage on 4 May 2021 two weeks after fertilization (2980.9 µg m^2 h^{-1} N_2O-N, Figure 6b).

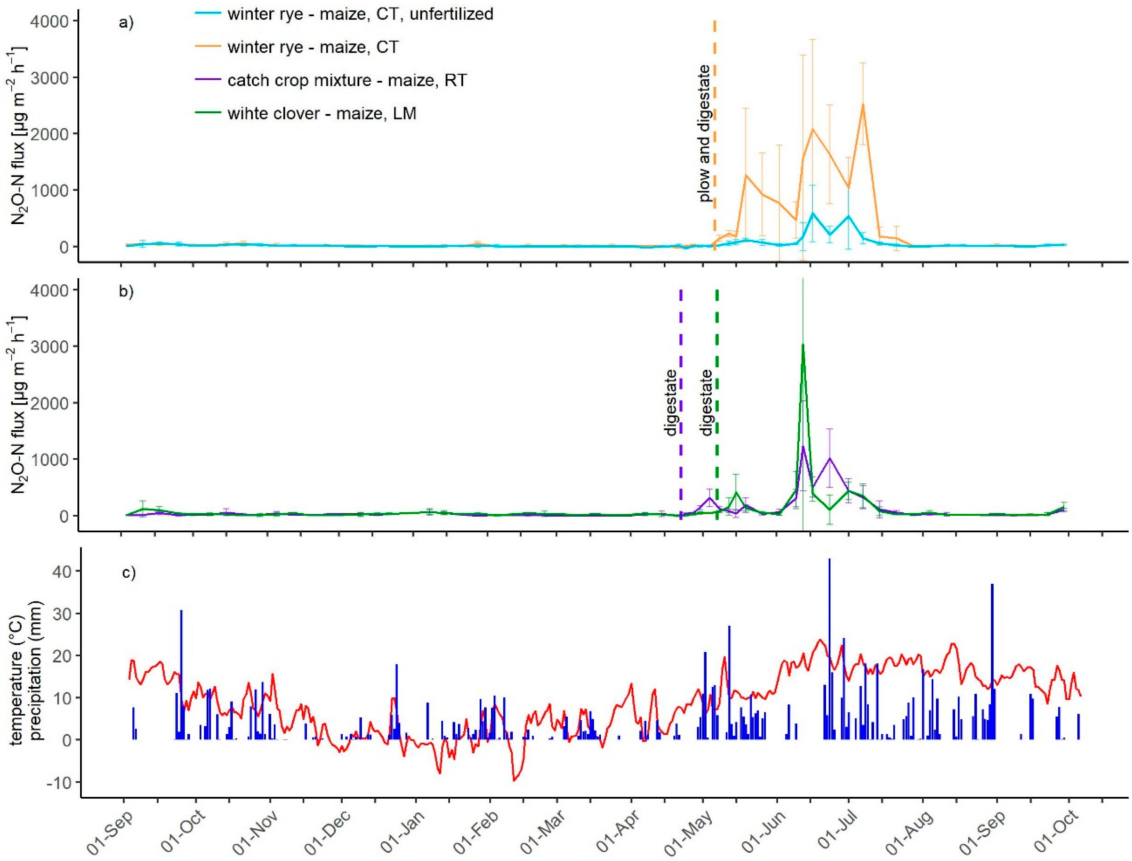

Figure 5. N_2O emissions for the treatments (**a**) winter rye—maize, CT, unfertilized and winter rye—maize, CT and (**b**) catch crop mixture—maize, RT, and white clover—maize LM from September 2019 until October 2020. Error bars illustrate the standard deviation. (**c**) Daily mean temperature and precipitation.

3.5. Cumulated N_2O Emissions and Yield-Related Emissions

In the following, the cumulative nitrous oxide emissions of the trial years are presented. These were calculated for different periods. The winter period refers to the time from established catch crop until maize sowing, the summer period refers to the time from maize sowing until harvest (see Appendix A Tables A2–A5). In addition, the total measurement period was presented for the years 2020 and 2021.

Significant differences were found between the different treatments and periods in all three years (Table 6), except for Summer 2021. In all summers, the cumulative N_2O emissions in the fertilized variants were higher than in the unfertilized winter rye—maize variant. Summer cumulative N_2O emissions of the fertilized winter rye—maize variant were 5.6 times higher (2019), 5.9 times higher (2020) and 2.4 times higher (2021) than those of the unfertilized rye—maize variant. Significant differences between these two variants could be demonstrated in 2019 and 2020. Among the fertilized variants, in 2019 the catch crop mixture—maize variant had the lowest cumulative N_2O emissions (2.04 kg ha^{-1}) compared to 5.65 kg ha^{-1} in the winter rye—maize variant, but the difference is not significant. In 2020, the catch crop mixture—maize variant and the white clover—maize variant also had lower N_2O emissions (6.29 and 6.65 kg ha^{-1}, respectively) than the winter rye—maize variant (19.05 kg ha^{-1}). In 2021, no significant differences could be detected. Due to the low number of nitrous oxide peaks in combination with the high scatter of the measured values, the variants are statistically not significantly different despite very different cumulative values.

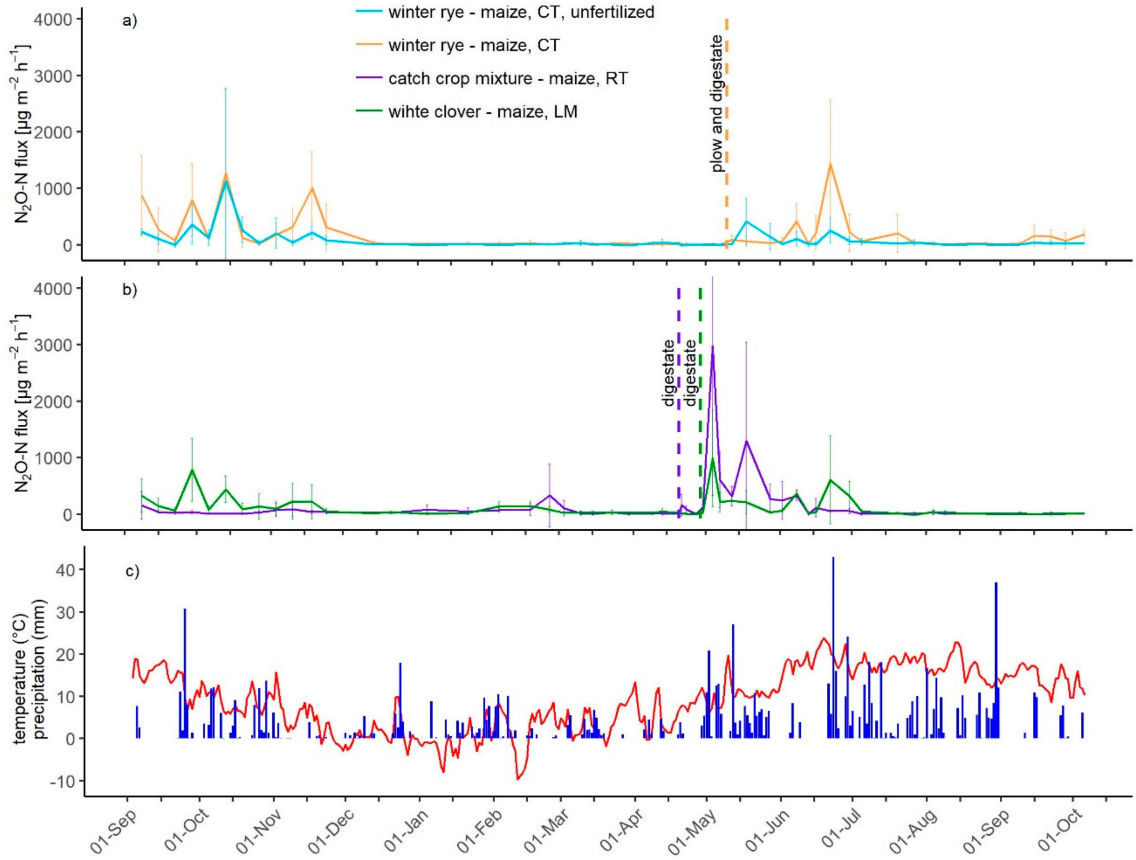

Figure 6. N_2O emissions for the treatments (**a**) winter rye—maize, CT, unfertilized and winter rye—maize, CT and (**b**) catch crop mixture—maize, RT, and white clover—maize LM from September 2020 until October 2021. Error bars illustrate the standard deviation. (**c**) Daily mean temperature and precipitation.

Winter emissions in 2019/2020 contributed only a small portion of total emissions (between 5 and 20.7% of total emissions). Nevertheless, significant differences between the variants could be detected. The highest winter N_2O emissions (1.57 kg ha^{-1}) were found in the white clover—maize variant and the lowest (0.70 kg ha^{-1}) in the unfertilized variant. In 2020/2021, there were nitrous oxide peaks in all variants, which are also reflected in the cumulative values. The highest emissions were calculated for the winter rye—maize variant with 9.3 kg ha^{-1}. These are higher than the summer emissions (5.6 kg ha^{-1}) and make up 62% of total emissions. The unfertilized winter rye—maize variant (5.0 kg ha^{-1}) and white clover—maize (living mulch, 5.1 kg ha^{-1}) also show significant nitrous oxide levels compared to the winter before. The lowest emissions in this period were detected in the catch crop mixture variant with 2.5 kg ha^{-1}. In relation to the entire measurement period, emissions were highest in both years in the winter rye—maize variant. The lowest emissions were detected in the unfertilized control. In 2020, white clover—maize is higher than catch crop—maize and the other way around in 2021. In 2021, the majority of nitrous oxide was measured in winter, with the exception of the catch crop mixture—maize variant. In 2020, only a very small proportion of emissions was measured in winter.

In general, N_2O fluxes were at high to very high levels, extremely high in the winter rye—maize variant in 2020.

The product-related emissions refer to the summer emissions from fertilization until harvest in order to enable a comparison of the cultivation systems. Due to high maize yields in 2019 and 2020, product-related emissions are very low except in winter rye—maize, CT (2020) yield-related emissions were significantly higher with 1.04 N_2O-N kg Mg^{-1} due to extremely high summer emissions. In 2021, a considerable part of the annual emissions was emitted in the winter; yield-related emissions were low.

Table 6. Cumulated N_2O-N emissions for the four variants and all three years. For 2020 and 2021, the emissions are additionally divided into winter and summer emissions. The yield-related emissions refer to the summer emissions from maize sowing and fertilization until harvest. Different letters indicate significant differences (Tukey test, $p \leq 0.05$).

Year	Treatment [a]	Total [b] N_2O-N kg ha^{-1}	Winter [c] N_2O-N kg ha^{-1}	Summer [d] N_2O-N kg ha^{-1}	Yield-Related [e] N_2O-N kg Mg^{-1}
2019	Winter rye—maize, CT, unfertilized			1.0 a	0.07 a
	Winter rye—maize, CT			5.6 c	0.27 a
	Catch crop mixture—maize, RT			2.0 ab	0.10 a
	White clover—maize, LM			4.9 bc	0.36 a
2020	Winter rye—maize, CT, unfertilized	4.0 a	0.7 a	3.2 a	0.30 a
	Winter rye—maize, CT	20.1 b	1.0 a	19.0 b	1.04 b
	Catch crop mixture—maize, RT	7.8 a	1.0 a	6.8 a	0.28 a
	White clover—maize, LM	8.2 ab	1.7 b	6.5 a	0.37 a
2021	Winter rye—maize, CT, unfertilized	7.3 a	5.0 ab	2.3 a	0.55 a
	Winter rye—maize, CT	15.0 b	9.3 b	5.6 a	0.86 a
	Catch crop mixture—maize, RT	10.2 ab	2.5 a	7.6 a	0.69 a
	White clover—maize, LM	9.4 ab	5.1 b	4.3 a	0.47 a

[a] CT = conventional tillage (plowing), RT = reduced tillage (mulching), LM = living mulch. [b] This period refers to the entire measurement campaign each year, starting in September with the catch crop and ending two weeks after harvesting (see Appendix A Tables A2–A5). [c] This period refers to the catch crop period from September until maize sowing (see Appendix A Tables A2–A5). [d] This period refers to the maize growing period from sowing until two weeks after harvest (see Appendix A Tables A2–A5). [e] Yield-related N_2O-N emissions in kg per Mg maize dry matter (related to maize from sowing to harvesting).

4. Discussion

4.1. Discussion of Methods

The investigations were carried out in a long-term field experiment, which was set up in 2005. Thus, long-term effects of different maize cultivation systems could be analysed. So far, N_2O fluxes of cultivation and fertilization systems have been analysed mainly in one-year or multi-year experiments [61]. These experiments cannot provide information about long-term effects. Especially the application of organic fertilizers (digestate) is expected to have long-term effects on N_2O fluxes, because the fertilizers contain organically bound carbon and nitrogen, which accumulates in the soil organic matter and is later mineralized [39,79]. Long-term experiments with organic farming systems are still rare [80,81]. The effects of crop rotation in combination with catch crops and tillage can also only be assessed with certainty considering the long-term effect [82,83].

The implementation of a nitrous oxide measurement campaign in an already established long-term experiment gave us the opportunity to analyse the effects of the cultivation systems, the digestate fertilization and the different management methods accumulated over many years.

In the trial, practically relevant maize cultivation systems were tested. Not only single factors were varied, but complete cropping systems were compared (system approach). An exception is the fertilized and unfertilized variant winter rye-maize. Here, only the fertilization is different, thus the effect of the digestate fertilization can be determined directly. The winter rye-silage maize crop rotation is increasingly grown in organic farming systems [84] and is dominant in conventional systems in the study region [85]. Non-legume catch crops such as winter rye can reduce N_2O emissions and nitrate leaching through nitrogen uptake compared to legume catch crops [57,86]. The catch crop mixture-maize and white clover-maize living mulch systems are designed primarily to reduce soil erosion and promote humus accumulation through permanent plant growth and soil cover [87]. While fall plowing followed by fallow before maize used to be the standard, intercropping with species-rich mixtures and subsequent mulch sowing of maize is becoming more common, especially on sites at risk of erosion. Not yet widely used in practice is the white clover–maize living mulch system. Measurement of nitrous oxide fluxes in crop field experiments has been conducted since the 1970s [88] and continues to gain importance due to the climate relevance of nitrous oxide emissions [9].

For researchers, the greatest difficulty is the multitude of factors influencing N_2O fluxes [89]. In addition, there is a high temporal and spatial variability of N_2O emissions [90,91] which is reflected in the large standard deviation of the repetitions in our trial. At 320 ppb, the atmospheric concentration of N_2O is 1000 times lower than that of CO_2, which requires high accuracy of measurement systems [92].

Small-scale soil differences (spatial variability of soil properties) in the experimental trial were investigated in more detail by Simon, 2021 [64]. In addition, the composition of the digestate varies considerably. It is also not possible to ensure uniform incorporation and distribution of the digestate in the trial plots, as the application is carried out using standard practice technology. The high spatial and temporal variability of emissions always plays a very important role in the measurement of nitrous oxide [93,94].

4.2. Discussion of Results

Due to the very high yields that can be achieved at this site under ecological conditions [63], the high amount of fermentation residues in this trial also results. The digestate applications (and the N-inputs) in the trial correspond to the theoretical accumulation of the respective system (system-conform fertilization)-derived from the yields of the energy crops taking into account the fermentation in the biogas process [63,64]. The moderate N balances (Table 4) also show that these N quantities can be well utilized by the plants. However, in farms with biogas plants, high N inputs with digestate are quite common practice [95,96].

The very high maize yields in the trial (up to more than 20 Mg ha^{-1}) demonstrate the high yield potential of the site. With sufficient nutrient supply-as in this trial through digestate fertilization-and intensive mechanical weed control, the maize yields almost reached the level of conventional farming [97].

In the experiment, extreme annual (weather) effects on yields and N balances were evident. In 2021, yields reached only about 50% of the level of previous years, therefore high N balances also occurred. This also had consequences for N_2O emissions, which were highest in 2021. It can be concluded that the increase in weather extremes would not only negatively affect yields, but could also lead to higher N balances and N_2O emissions.

When evaluating the cropping systems, it should be noted that the catch crop winter rye also provides a substantial yield (substrate for the biogas plant), while the other catch crops only serve as green manure. The winter rye intercrop can also utilize manure in the fall, and absorb significant amounts of nitrogen in the winter half-year, minimizing N losses during this period. However, winter rye must achieve sufficient biomass development by harvest, resulting in later maize seeding (2–10 May in the trial versus 23–27 April in variant catch crop mixture-maize). The temporarily high ammonium and nitrate values can have different causes; an influence of the soil sampling method cannot be excluded. Composite samples of the variants were taken and digestate can get into the sample. An increase in soil mineral nitrogen after tillage and fertilization was measured in the trial. N_2O emissions originate mainly in the upper soil layers [98]. The incorporation of the fermentation residues with the plow directly after fertilizer application shifts the fermentation residues to deeper soil layers than in the white clover-maize variant. The use of the plow generally leads to a mineralization boost [33].

The N uptake from maize does not occur directly after sowing and fertilization. Therefore, at the time of sowing the content of soil mineral nitrogen is high due to mineralization and fertilization in the spring. The main nutrient requirement is in the period from late June to mid-August, i.e., relatively late in the vegetation phase [99]. The available nitrogen is therefore not completely taken up by the plants and is subject to microbial turnover processes that produce nitrous oxide. This is particularly evident in the winter rye variant. In this variant, a clear increase in N_2O fluxes after fertilization is evident in all years. This continues until July. At this time, the maize is in the BBCH stage of shooting (30–39). In the white clover variant, the nitrogen application of the fertilizer could be partially removed by the clover and thus reduced the emissions, but also the yields. Very high N_2O fluxes were measured in the field experiment, and cumulative N_2O emissions were also very high. This confirms earlier measurement results at the same experimental site [100,101] and at the nearby experimental site Scheyern [102].

The specific soil-climatic conditions favour high N_2O fluxes. The soils in the trial are susceptible to compaction and capping and are poorly aerated [64,103]. In studies by Peter et al. (2013), cumulative N_2O emissions were about twice as high as calculated with emission factors according to IPCC or simulated with the DNDC model [104].

Compared to the unfertilized variant (winter rye-maize), the digestate fertilization resulted in a remarkable increase in N_2O-N fluxes from 1.0 to 5.0 kg ha^{-1} in 2019, from 3.3 to 19.0 kg ha^{-1} in 2020, and 2.3 to 7.6 kg ha^{-1} N_2O-N in 2021, respectively. The higher precipitation in the vegetation season 2020 (compared to 2019) apparently favoured high N_2O fluxes. One reason for the much higher N_2O fluxes in the fertilized variants (compared to the unfertilized plots) is the different fertilization of the experimental plots over 15 years. Results from Levin 2021 show significant increases in soil organic carbon after fertilization with biogas digestate in the same trial [63]. The intense N_2O fluxes occurred predominantly during the periods when high soil ammonium and nitrate levels were also observed.

In all three experimental years, N_2O emissions occurred predominantly in the spring after fertilization and maize sowing until July within 2 to 3 months. After that, the emissions decreased and remained at a low level until the post-harvest period in September/October. One explanation for the drastic decrease in N_2O fluxes is the high N uptake by the growing maize stands. In addition, soil water content decreased significantly during the summer. Winter catch crops can have positive effects, such as erosion control, nutrient conservation and protection from nitrate leaching, humus accumulation, weed suppression, and promotion of microbial activity [17]. Potential disadvantages include additional water use, additional field operations, and possible promotion of diseases or pests [105]. Species composition, legume content, C:N ratio, and protein content of catch crops affect N_2O fluxes, but these effects are site-dependent and highly variable [17,58]. Incorporating legume-rich catch crops into the soil can increase N_2O emissions [16,106,107].

Intensive tillage by plowing in spring at relatively high soil temperatures and soil moisture resulted in nitrogen mineralization (increase in ammonium and nitrate stocks) and elevated N_2O emissions in the winter rye-maize variants. In 2021, the catch crop-maize variant also shows high N_2O emissions. The rainfall events and the large amount of organic mulch combined with digestate fertilization created good conditions for denitrification.

Leguminous catch crop mixtures with subsequent mulch seed offer potential for organic maize cultivation. Less tillage is required, the soil is additionally supplied with N and C and has a good water holding capacity. However, they can lead to increased N_2O emissions by an additional N input [107,108] and promote denitrification [109]. The effects of the intercrops on the N_2O emissions are very complex and therefore discussed controversially [17,107,108]. However, after catch crop dieback following the first severe frost, mineralization of catch crop biomass begins (increase in ammonium and nitrate levels [110], and N_2O fluxes may occur during subsequent freeze-thaw cycles [111]. However, this was not observed under the experimental conditions.

Pelster et al. (2011) found a positive effect of intercropping and reduced tillage on N_2O emissions [20]. However, on light and well-aerated soils, no difference could be shown between reduced and intensive tillage in terms of nitrous oxide emissions [98].

At the Viehhausen experimental site with rather heavy soils and a high N_2O emission potential [112], the highest emissions were measured in the plowed variant compared to reduced tillage. High temperatures in combination with plowing and digestate fertilization led to a mineralization boost and increased N_2O emissions.

Cultivation systems with living mulch are still very controversial discussed and not common practice. Although the use of living mulch has many ecological advantages, these must be weighed against the possible yield losses [113]. Our trial also showed a significant yield loss in all years compared to the other two fertilized variants. Of particular importance is the strong suppression of white clover before sowing maize in order to give the maize, which is not very competitive when young, the opportunity to achieve good juvenile development [54].

5. Conclusions and Outlook

After three experimental years with very different weather conditions, statements can be made for dry and very wet conditions; more frequent weather extremes can be expected in the future [114]. Yields, N balances, and N_2O emissions are significantly different in the investigated variants, suggesting that intercropping, fertilization, and tillage have an effect on yield formation, soil nitrogen turnover, and denitrification.

Based on our results, hypothesis 1 (*Fertilization with digestate will causes a significant increase in N_2O fluxes in organic maize cultivation)* can be confirmed. Fertilization with digestate resulted in significantly higher N_2O emissions in all variants compared to the unfertilized control variant.

Hypothesis 2 (*Conventional tillage prior to maize sowing stimulates soil N mineralization and increases soil nitrate levels as well as N_2O emissions*) can be confirmed.

The winter rye-maize variant produces high yields but has to be evaluated very critically due to high N_2O fluxes. There is a need for optimization in this variant. Fertilization and tillage intensity should be reduced to decrease N mineralization and N_2O emissions

Hypothesis 3 (*Conservation tillage decreases N mineralization and N_2O fluxes (compared to plow tillage) without yield reduction*) can be confirmed.

The investigations show that soil-conserving maize cultivation in the variant catch crop mixture–maize can be recommended to practice under the given site conditions. This variant results in very high dry matter yields, low N balances (Table 4) and significantly lower N_2O emissions than the winter rye-maize variant.

Hypothesis 4 (*Maize cultivation in living mulch systems results in low N_2O emissions, but also in significant yield losses*) can be confirmed.

The white clover–maize (living mulch) variant is associated with high yield risks (see Table 4), caused by the competition of maize with white clover and weeds. In terms of soil protection and N_2O fluxes, this variant is equivalent to the catch crop mixture–maize variant.

In this publication, we presented results on organic maize cropping systems from a long-term field experiment. This experiment contains variants with different intercrops, energy crop species, cash crops, crop rotations, and digestate application rates (see Figure A2). In further publications we will present corresponding results of other crop types (e.g., winter wheat, fertilized with different amounts of digestate) from this experiment.

Furthermore, we will calculate greenhouse gas balances based on the experimental data, including not only N_2O emissions but also soil C sequestration and CO_2 emissions from fossil energy use.

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

Appendix A

Table A1. Soil properties at 0–30 cm depth in the experimental station Viehhausen, long-term field experiment "energy crops and crop rotation trial".

Year	Treatment	SOC %	N %	C/N	pH	Bulk Density g/cm^3
2019	Winter rye—maize	1.17	0.12	9.7	6.66	1.31
	Winter rye—maize	1.30	0.13	9.9	6.71	1.29
	Catch crop mixture—maize	1.13	0.12	9.3	6.42	1.26
	White clover—maize	1.24	0.14	9.2	6.38	1.26
2020	Winter rye—maize	1.10	0.12	9.1	6.21	1.37
	Winter rye—maize	1.11	0.13	8.8	6.39	1.31
	Catch crop mixture—maize	1.18	0.12	9.5	6.7	1.4
	White clover—maize	1.23	0.14	9.0	6.18	1.4
2021	Winter rye—maize	1.06	0.11	9.5	5.77	1.4
	Winter rye—maize	1.17	0.12	9.4	5.97	1.43
	Catch crop mixture—maize	1.15	0.13	9.1	5.87	1.33
	White clover—maize	1.17	0.13	9.1	5.9	1.39

Figure A1. Split chamber design for measurements of nitrous oxide emissions in high plant stands such as maize according to Olfs et al. 2018.

Table A2. Soil and crop management for winter rye—maize with conventional tillage and digestate fertilization.

Crop Management	2018/19 Date	2019/20 Date	2020/21 Date
Winter rye—maize, conventional tillage, fertilized with digestate			
Tillage (cultivator) Tillage (cultivator) Tillage (cultivator) Tillage (cultivator)	19 July 2018 6 September 2018 18 September 2018	30 July 2019 12 August 2019 30 August 2019 18 September 2019	30 July 2020 12 August 2020 18 September 2020
Seedbed preparation (circular harrow)			18 August 2020
Sowing of winter rye (seed drill)	18 September 2018	19 September 2019	18 August 2020
Harvest of winter rye (chopper)	2 May 2019	5 May 2020	10 May 2021
Digestate application (slurry tanker with trailing hoses)	2 May 2019	6 May 2020	10 May 2021
Tillage (plow)	2 May 2019	6 May 2020	10 May 2021
Seedbed preparation (circular harrow)	2 May 2019	6 May 2020	10 May 2021
Sowing of maize (presicion drilling)	2 May 2019	6 May 2020	11 May 2021
Mechanical weed control (harroweeder) Mechanical weed control (harroweeder)	18 May 2019 6 June 2019	16.05.2020	
Mechanical weed control (hoe) Mechanical weed control (hoe)	14 June 2019	13 June 2020 24 June 2020	7 June 2021 12 June 2021 21 June 2021 28 June 2021
Harvest of maize (maize chopper)	17 September 2019	17 September 2020	11 October 2021

Table A3. Soil and crop management for winter rye—maize with conventional tillage, unfertilized.

Crop Management	2018/19 Date	2019/20 Date	2020/21 Date
Winter rye—maize, conventional tillage, no fertilizer			
Tillage (cultivator) Tillage (cultivator) Tillage (cultivator) Tillage (cultivator)	19 July 2018 06 September 2018 18 September 2018	30 July 2019 12 August 2019 30 August 2019 18 September 2019	30 July 2020 12 August 2020 18 September 2020
Seedbed preparation (circular harrow)			18 September 2020
Sowing of winter rye (seed drill)	18 September 2018	19 September 2019	18 September 2020
Harvest of winter rye (chopper)	2 May 2019	5 May 2020	10 May 2021
Tillage (plow)	2 May 2019	6 May 2020	10 May 2021
Seedbed preparation (circular harrow)	2 May 2019	6 May 2020	10 May 2021

Table A3. *Cont.*

Crop Management	2018/19 Date	2019/20 Date	2020/21 Date
Sowing of maize (presicion drilling)	2 May 2019	6 May 2020	11 May 2021
Mechanical weed control (harroweeder) Mechanical weed control (harroweeder)	18 May 2019 6 June 2019	16 May 2020	
Mechanical weed control (hoe) Mechanical weed control (hoe)	14 June 2019	13 June 2020 24 June 2020	7 June 2021 12 June 2021 21 June 2021 28 June 2021
Harvest of maize (maize chopper)	17 September 2019	17 September 2020	11 October 2021

Table A4. Soil and crop management for catch crop mixture—maize with reduced tillage and digestate fertilization.

Catch crop mixture—maize Reduced tillage Fertilized with digestate			
Tillage (cultivator)	19 July 2018	3 July 2019	30 July 2020
Tillage (plow)	1 August 2018	8 August 2019	10 August 2020
Seedbed preparation (circular harrow)			11 August 2020
Sowing of catch crop mixture (seed drill)	1 August 2018 [a]	9 August 2018 [b]	11 August 2020 [c]
Seedbed preparation (bed roller)	4 August 2018	30 October 2019	
Biomass cut	16 October 2018	30 October 2019	26 October 2020
Tillage (disc harrow)		31 March 2020	
Digestate application (slurry tanker with trailing hoses)	24 April 2019	22 April 2020	20 April 2021
Seedbed preparation (disc harrow)	24 April 2019	22 April 2020	20 April 2021
Seedbed preparation (circular harrow)	25 April 2019		27 April 2021
Sowing of maize (presicion drilling)	25 April 2019	23 April 2020	27 April 2021
Mechanical weed control (harroweeder)	13 May 2019 18 May 2019	7 May 2020	
Mechanical weed control (rotary cultivator)			31 May 2021
Mechanical weed control (hoe)	4 June 2019 14 June 2019	29 May 2020 13 June 2020	11 June 2021
Harvest of maize (maize chopper)	17 September 2019	17 September 2020	11 October 2021

[a] 2018: 150 kg *Vicia Faba*, 100 kg *Pisum sativum*, 30 kg *Vicia sativa*, and 3 kg *Helianthus annuus*; [b] 2019: 150 kg *Vicia Faba*, 100 kg *Pisum sativum*, 20 kg *Avena strigose*, and 3 kg *Helianthus annuus*; [c] 2020: 110 kg *Vicia Faba*, 73 kg *Pisum sativum*, 15 kg *Avena strigose*, and 2 kg *Helianthus annuus*.

Table A5. Soil and crop management for living mulch white clover—maize with digestate fertilization.

White clover—maize Living mulch Fertilized with digestate			
Tillage (cultivator)	19 July 2018	30 July 2019	30 July 2020 12 August 2020
Tillage (plow)	3 August 2018	8 August 2019	19 August 2020
Tillage (harrow)	3 August 2018		19 August 2020
Sowing of living mulch (seed drill)	3 August 2018 [a]	09 August 2019 [b]	19 August 2020 [c]
Tillage (roller)	4 August 2018		
Mulching	22 October 2018		
Biomass cut	16 October 2018	30 October 2019	26 October 2020
Tillage (cultivator)	23 October 2018		
Tillage (rotary cultivator)	2 April 2019	31 March 2020	16 April 2021
digestate application (slurry tanker with trailing hoses)	2 May 2019	7 May 2020	29 April 2021
Tillage (rotary cultivator)	2 May 2019	7 May 2020	29 April 2021
Sowing of maize (presicion drilling)	2 May 2019	7 May 2020	29 April 2021
Mechanical weed control (harroweeder)	6 May 2019 13 May 2019 18 May 2019 4 June 2019	16 May 2020	04 May 2021
Mechanical weed control (mulching)		28 May 2020	31 May 2021
Mechanical weed control (rotary cultivator)	4 June 2019	29 May 2020	31 May 2021
Mechanical weed control (mulching)			9 June 2021
Mechanical weed control (rotary cultivator)			9 June 2021
Mechanical weed control (harroweeder)			9 June 2021
Mechanical weed control (hoe)		13 June 2020	12 June 2021 21 June 2021
Mechanical weed control (harroweeder)	6 June 2019		
Mechanical weed control (mulching)		23 June 2020	
Harvest of maize (maize chopper)	17 September 2019	17 September 2020	11 October 2021

[a] 2018: 15 kg *Trifolium repens* and 28 kg *Avena strigose*; [b] 2029: 15 kg *Trifolium repens* and 25 kg *Avena strigose*; [c] 2020: 15 kg *Trifolium repens* and 25 kg *Avena strigosea*.

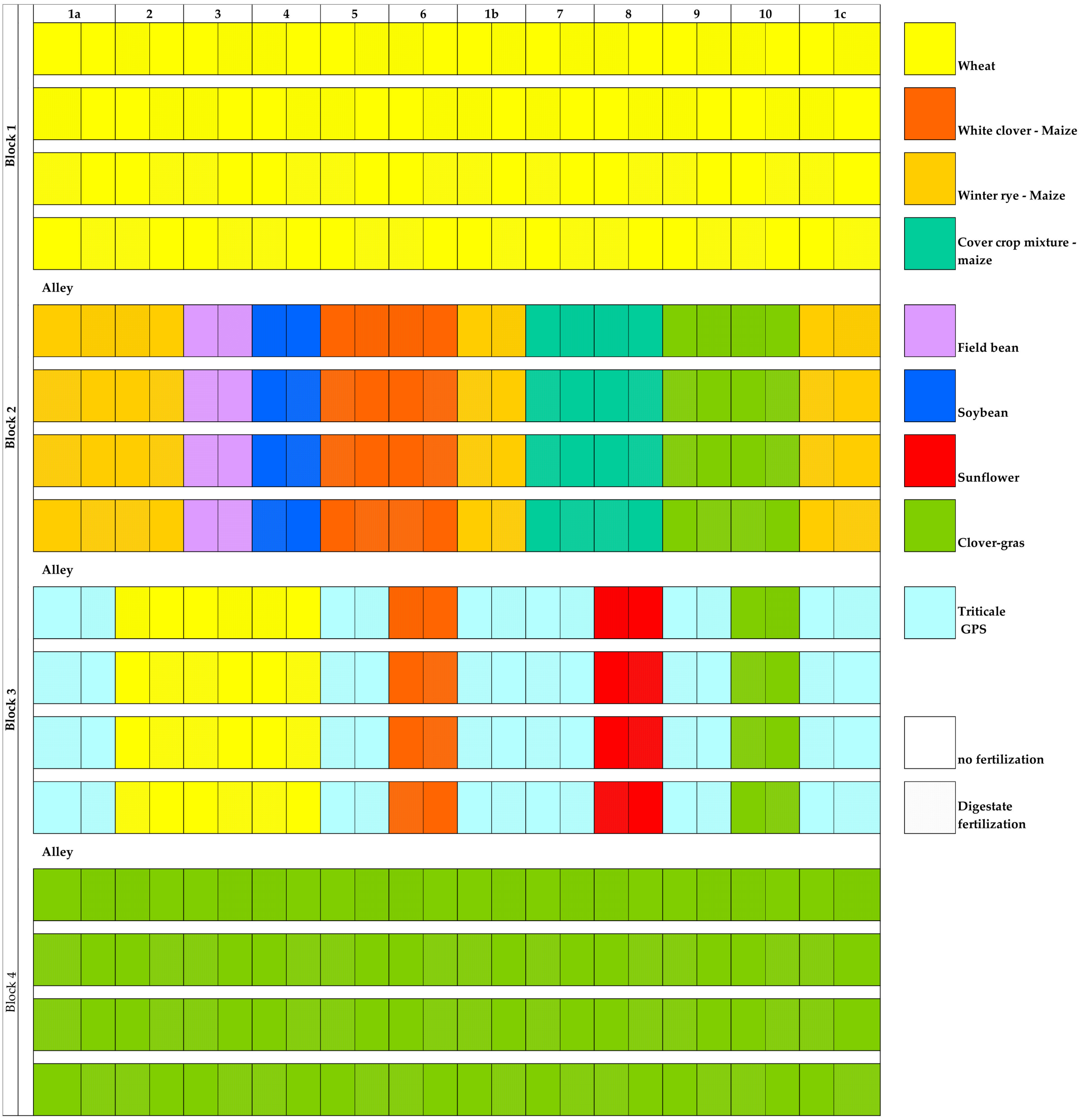

Figure A2. Field trial plot plan 2019. For dimensions, see text. Crop rotations are indicated in the top row.

Appendix B

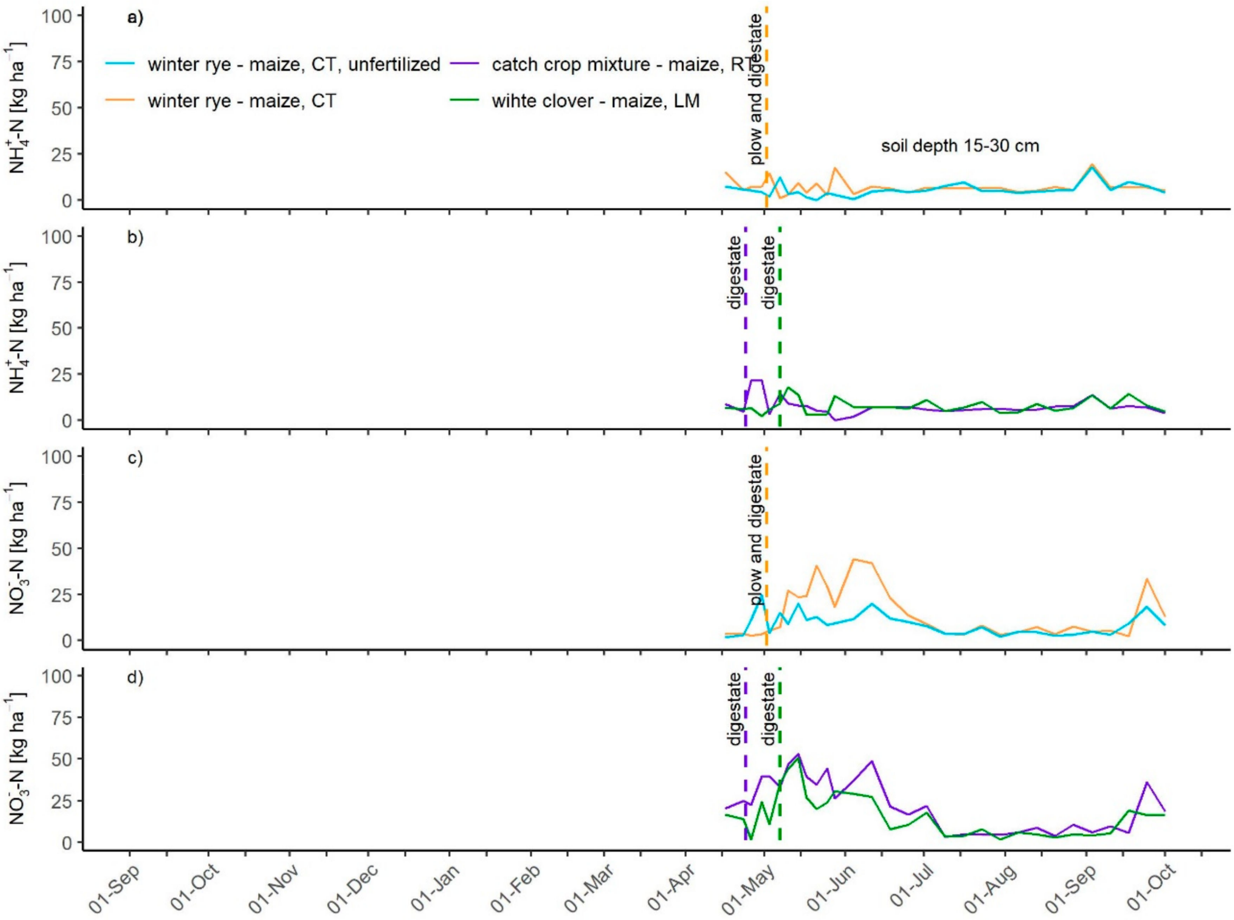

Figure A3. Ammonium and nitrate dynamics of all four variants related to the top soil layer of 15–30 cm from April 2019 to September 2019. (**a**) Ammonium values in both winter rye—maize variants; (**b**) ammonium values in catch crop mixture—maize with reduced tillage and white clover—maize with living mulch; (**c**) nitrate values in both winter rye—maize variants; and (**d**) nitrate values of crop mixture—maize with reduced tillage and white clover—maize.

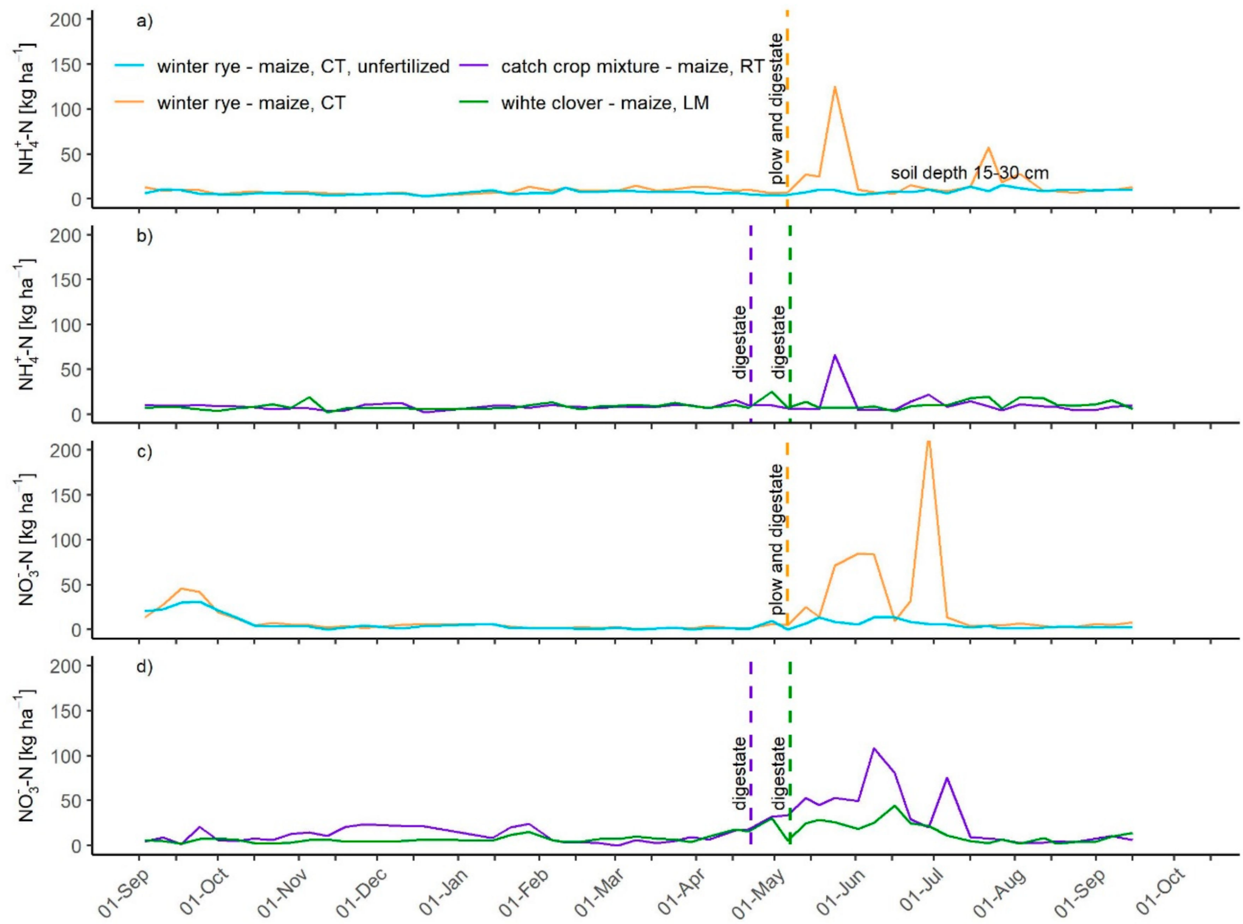

Figure A4. Ammonium and nitrate dynamics of all four variants related to the top-soil layer of 15–30 cm from September 2019 to September 2020. (**a**) Ammonium values in both winter rye—maize variants; (**b**) ammonium values in catch crop mixture—maize with reduced tillage and white clover—maize with living mulch; (**c**) nitrate values in both winter rye—maize variants; and (**d**) nitrate values of crop mixture—maize with reduced tillage and white clover—maize.

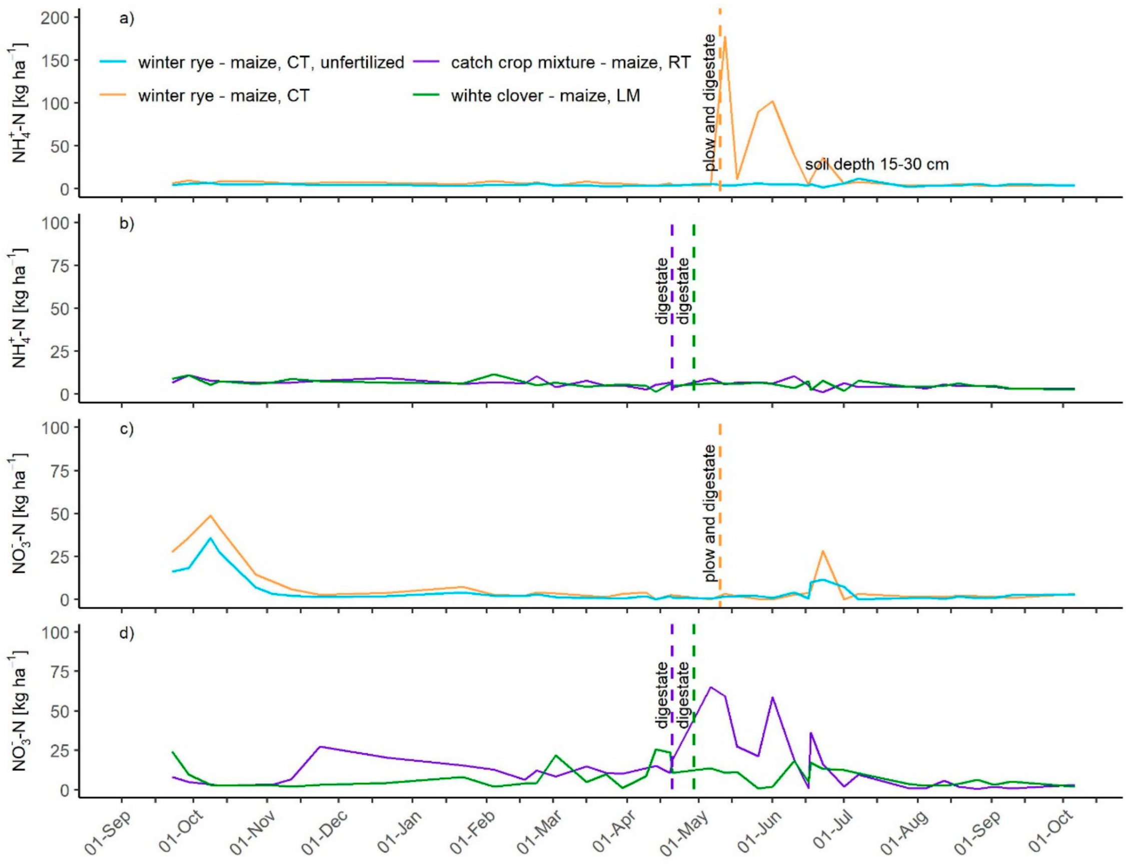

Figure A5. Ammonium and nitrate dynamics of all four variants related to the top-soil layer of 15–30 cm from September 2020 to October 2021. (**a**) Ammonium values in both winter rye—maize variants; (**b**) ammonium values in catch crop mixture—maize with reduced tillage and white clover—maize with living mulch; (**c**) nitrate values in both winter rye—maize variants; and (**d**) nitrate values of crop mixture—maize with reduced tillage and white clover—maize.

Figure A6. Soil moisture in all three years and treatments of investigation. Gravimetric water content in % was analyzed from weekly soil samples. (**a**) 2019, (**b**) 2020, and (**c**) 2021.

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