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Integrated rather than organic farming history facilitates soil nitrogen turnover and N$_2$O reduction in a green rye – silage maize cropping sequence

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Abstract

Soil gross mineral N production and consumption processes are crucial regulators of plant productivity and N loss from croplands. Substituting synthetic fertilizers by integrating legumes in cultivation systems is common in organic farming, but research on its long-term impact on dynamics of gross soil N transformation and associated environmental N loss is scarce. In particular, studies at a temporal resolution that allows for a mechanistic understanding of long-term effects of organic farming are missing. Therefore, we determined gross N turnover rates of ammonification, nitrification, and ammonium and nitrate immobilization at monthly temporal resolution during a full green rye-maize cropping sequence. Measurements were carried out at sites with same pedo-climatic background but organic farming (OF) and integrated farming (IF) history. During green rye growing, N turnover rates for OF and IF were low and not significantly different, likely owing to low temperatures. During silage maize growing, IF exhibited significantly higher average N turnover rates of 1.86, 4.46, and 5.57 mg N kg^{-1} dry soil d^{-1} for gross ammonification, ammonium immobilization, and nitrate immobilization, respectively, compared to OF values of 1.11, 1.80, and 2.90 mg N kg^{-1} dry soil d^{-1}. The significantly higher N turnover rates were likely due to higher soil organic C, N and microbial biomass which result from different long-term management practices. Especially the increased immobilization potential on the IF site contributed to significantly lower area-scaled N$_2$O emissions (1.45 vs. 4.36 kg N ha^{-1}) during periods of high nitrification. This shows that for low SOC soils, integrated farming history with high C return enhances soil N cycling and reduces the risk of N losses in the form of N$_2$O emission.

Keywords Gross N turnover · N$_2$O emission · Isotope pool dilution · Farming systems · Green rye – silage maize · Legumes

Introduction

Soil microbial nitrogen (N) turnover comprises the processes N mineralization, N immobilization, nitrification, and denitrification (Robertson and Groffman 2007). Nitrogen mineralization converts soil organic matter (SOM) into ammonium (NH$_4^+$), which is either taken up by plants, immobilized by microorganisms or transformed into nitrate (NO$_3^-$) during the process of nitrification. Nitrate is either taken up by plants, immobilized by microorganisms, or transformed into gaseous N species via several intermediate steps during the process of denitrification of which the terminal product is molecular nitrogen (N$_2$).

Consequently, the interplay of the N turnover processes mineralization / nitrification and NH$_4^+$ / NO$_3^-$ immobilization affects N availability for plant uptake (Hu et al. 2019),

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but also the vulnerability of an ecosystem towards N losses to the environment in the form of reactive N compounds, mainly ammonia (NH_3), nitrate (NO_3^-), nitrous oxide (N_2O), and nitric oxide (NO) (Butterbach-Bahl et al. 2011; Zöll et al. 2016). Release of N to the environment is particularly problematic in agricultural soils which receive high loads of N fertilizers to optimize crop yield.

In this context, long-term exclusive synthetic fertilizer use in conventional agricultural systems was found to promote gross ammonification but not NH_4^+ immobilization, resulting in increased gross nitrification and the risk of N losses (Dai et al. 2017). Similarly, Wang et al. (2020b) reported that the long-term application of synthetic fertilizer decreased total soil N (TN) but increased denitrifier abundance. In contrast, long-term organic fertilizers and even more a mix of organic and mineral fertilizer promoted mineralization-immobilization N turnover which reduced N losses and increased the N accessibility to plants (Gardner and Drinkwater 2009; Dai et al. 2017; Wang et al. 2023b). N availability for plants and crop N uptake due to increased mineralization was also observed for extended crop rotation durations of 3–4 years receiving organic fertilizers compared to 2-year rotations with mineral N fertilizers (Osterholz et al. 2017). Consequently, more diversified cropping systems combined with organic fertilization may have the potential to reduce N losses to the environment, but detailed field measurements are still scarce.

Diversifying crop rotations by integrating legumes has been discussed as a means of "agro-ecological" intensification in order to reduce fossil energy consumption for fertilizer production used in agriculture (Reza and Sabau 2022). Legumes may, thus, represent an approach for food production at reduced environmental costs by providing high nutritious yields while minimizing fertilizer input (Costa et al. 2021), with additional benefits for improved soil structure, SOM content, nutrient availability, and pest control (Böhm et al. 2020). Regarding N turnover rates, Lama et al. (2020) concluded that legumes boosted the rates of gross N mineralization, microbial NH_4^+ consumption, and inorganic N immobilization rates, presumably as a result of greater N availability via biological N_2 fixation.

Regarding the determination of gross ammonification and nitrification, the "isotope pool dilution" (IPD) method (Kirkham and Bartholomew 1954; Davidson et al. 1991) is well established, but different methods for the determination of immobilization rates are available. The IPD approach is the most frequently used approach but may be affected by substrate stimulation due to the N addition inherent to the method. To avoid this effect, budget methods such as the "reformed difference method" (RDM, Hu et al. 2019) are on the rise, but there is a lack of comparisons between IPD and RDM immobilization, especially during the course of the growing season.

Additionally, studies involving directly adjacent sites with identical pedo-climatic conditions but different long-term management histories are scarce. Most gross N turnover studies to date have focused on short-term inquiry due to the time-intensive nature of quantifying gross N turnover rates. Thus, studies with regular (for instance monthly) assessments of N turnover rates are mostly missing (Hu et al. 2019), which highlights the need for research investigating the effects of different farming history on soil gross N turnover rates for the whole growing season (Böhm et al. 2020) and their impacts on environmental N losses.

For these reasons, this study compares gross N turnover rates determined approximately monthly during a whole growing season of two directly adjacent sites representing different farming systems and management histories, but for the same crop sequence. While one site reflects an organic farming (OF) approach, in which inputs through mineral fertilizers are replaced through legume cropping, and organic fertilizers are used only sporadically, the second site reflects an integrated farming (IF) approach that combines mineral and organic fertilizer applications. The objectives of this study are (i) to characterize the dynamics and magnitude of gross N turnover rates during an entire growing season in high temporal resolution, (ii) to assess different approaches to determine gross microbial N immobilization, (iii) to investigate the impact of management history (IF vs OF) on soil gross N turnover rates and N_2O emissions, and (iv) to assess the environmental controls of gross N turnover rates.

Materials and methods

Study sites, management history and initial soil sampling

The experiment was conducted (50°21′28.8"N 8°15′47.4"E, elevation 310 m a.s.l) close to the Research Farm Gladbacherhof, Justus-Liebig University Giessen, Germany. The region belongs to the cold temperate climatic zone, with annual precipitation and average temperature amounting to 655 mm and 9.3 °C, respectively. The soil is classified as a Haplic Luvisol (World Reference Base), with an effective rooting depth of at least 110 cm (Schulz et al. 2014). The experimental area encompasses two adjacent sites with an area of ca. 1.0 ha each. Decisive factors for selection of these specific sites were the different management history, the identical pedo-climatic conditions and the access to mains power for monitoring N_2O emission in high temporal resolution.

During the period 2012 to 2020, one of the sites was managed organically with a specific focus on minimizing external N inputs. Consequently, N is largely derived from belowground allocation of N through biological N_2

fixation by legumes in the crop rotation and occasional supplements of organic cattle slurry (Table 1), so that the site is referred to as organic farming (OF). The other site was managed conventionally, following a soil C-building strategy, with synthetic N fertilizer being applied every year and organic fertilizer (sewage sludge or solid part of separated slurry) being applied approximately every second year. For this reason, the latter site is referred to as integrated farming (IF) site. On average, the OF site received 200 kg C ha^{-1} y^{-1} and 23 kg N ha^{-1} y^{-1}, while the IF site received 410 kg C ha^{-1} y^{-1} and 184 kg N ha^{-1} y^{-1}. Tillage practice was consistent across the two sites, with a depth of 20 cm.

To investigate potential differences in soil properties resulting from the different management histories, soil samples from both sites were collected at four different locations and at two depths (0–10 cm and 10–30 cm) at the beginning of the field trial. The soil physicochemical properties pH, soil organic C (SOC), total N (TN), soil organic C to total N ratio (C:N), bulk density (BD), and soil texture were determined (Table 2, results section). Soil pH was measured by a pH meter (metrohm, inolab 7310, wtw, Germany) after the dilution of soil samples in distilled water at a ratio of 1:5 (soil to water, Dannenmann et al. 2016).

The soil samples used for SOC and TN analysis were mixed, ground using a mixer mill (Retsch, MM301, Haan,

Table 1 Annual C and N input of organic farming (OF) and integrated farming (IF) sites for the period 2012–2020, i.e., prior to the field experiment. All crops were mowed from both sites with same machinery. Crop residues were returned to IF site but not to OF site. Tillage to a depth of 20 cm was uniformly executed at both sites, OF and IF. In case of missing documentation, gaps were filled based on recommended and typical practice for the area and literature values

Year	Organic farming (OF)				Integrated farming (IF)			
	Crop	Input type	kg C ha^{-1} y^{-1}	kg N ha^{-1} y^{-1}	Crop	Input type	kg C ha^{-1} y^{-1}	kg N ha^{-1} y^{-1}
2012	silage maize	cattle slurry	900	105	winter wheat	mineral N		180
2013	winter spelt				winter wheat	mineral N		180
2014	red clover & grass				winter wheat	mineral N		110
						sewage sludge	795	123
2015	red clover & grass				silage maize	mineral N		110
						separated slurry	837	57
2016	winter wheat				winter wheat	mineral N		180
2017	silage maize	cattle slurry	900	105	winter wheat	mineral N		110
						sewage sludge	373	60
2018	winter wheat				winter rye	mineral N		100
						separated slurry	837	57
2019	alfalfa and grass				winter wheat	mineral N		180
2020	alfalfa and grass				silage maize	mineral N		140
						sewage sludge	848	71
Mean			200	23			410	184

Table 2 Soil properties at the start of the experimental period in 2021 of the organic farming (OF) and integrated farming (IF) sites. Abbreviations SOC, TN, C:N and BD denote soil organic C content, total N content, C to N ratio and bulk density. Values are given as mean $\pm$ standard error (SE, $n = 4$)

Treatment	Depth [cm]	pH	SOC [%]	TN [%]	C:N [-]	BD [g cm^{-3}]	Texture analysis [%]		
							sand	silt	clay
OF	0–10	5.43 $\pm$ 0.05[a]	1.04 $\pm$ 0.12[a]	0.13 $\pm$ 0.01[a]	8.00	1.45 $\pm$ 0.01	10.20	68.30	21.50
IF	0–10	6.84 $\pm$ 0.07[b]	1.58 $\pm$ 0.07[b]	0.18 $\pm$ 0.01[b]	8.77	1.36 $\pm$ 0.02	8.20	67.10	24.70
OF	10–30	5.33 $\pm$ 0.06[a]	0.84 $\pm$ 0.19	0.11 $\pm$ 0.02	7.63	1.43 $\pm$ 0.03	11.60	65.10	23.30
IF	10–30	6.34 $\pm$ 0.25[b]	1.08 $\pm$ 0.08	0.14 $\pm$ 0.01	7.71	1.32 $\pm$ 0.01	9.20	68.30	22.50
Weighted Average (OF)	0–30	5.36 $\pm$ 0.06[a]	0.91 $\pm$ 0.17[a]	0.12 $\pm$ 0.02[a]	7.81	1.44 $\pm$ 0.02	11.13	66.17	22.70
Weighted Average (IF)	0–30	6.51 $\pm$ 0.19[b]	1.25 $\pm$ 0.08[b]	0.15 $\pm$ 0.01[b]	8.24	1.33 $\pm$ 0.02	8.87	67.90	23.23

Superscript letters indicate significant differences (t-test, $p < 0.05$) between sites OF and IF

Germany) and packed in tin capsules. Then the SOC and TN content were determined by elemental analysis coupled to mass spectrometry (Delta Plus XP; Thermo, Bremen, Germany) as described by Dannenmann et al. (2016). For BD, soil samples were taken using core cutters of 100 cm^3 (Khan et al. 2020). For texture analysis soil samples were collected from 10 randomly distributed locations across each of both sites (OF, IF), pooled, air-dried for three days, sieved (2 mm) and analysed by a commercial laboratory (AGROLAB, Breslauer Str. 60, 31,157 Sarstedt, Germany).

Management operations during field experiment

The field experiment started in October 2020 with the establishment of a green rye–silage maize cropping sequence. In order to avoid bias of measurements between sites caused by different crop, fertilizer amounts and fertilizer types, the same crops were cultivated and the same fertilizer amounts and types were used simultaneously on both sites in the experimental year 2021 (Fig. 1). Green rye was sown with a seed density of 300 kernels m^{-2} using a drilling device to plant the seeds at a depth of 3 cm. On April 8th, 25 m^3 ha^{-1} of cattle slurry was applied on both sites as a top-dressing application for green rye using a dragging hose technique, representing 88 kg N ha^{-1} and 750 kg C ha^{-1}. On May 31st, after the green rye harvest, 20 m^3 ha^{-1} of cattle slurry (74.8 kg N ha^{-1} and 600 kg C ha^{-1}) was applied as a pre-sowing fertilisation on both sites and subsequently incorporated by one pass of a disc harrow followed by a rotary tiller. Subsequently, silage maize was sown at a plant density of 90,000 plants ha^{-1}. Green rye and silage maize were harvested using a commercial forage harvester on May 10th and September 30th, respectively.

Measurements of gross N turnover rates

At the beginning of the experiment, five subplots (1 m^2) randomly distributed over each site were established where soil samples for determination of N turnover rates were collected from a 0.1 m by 0.1 m area. Since the topsoil (up to 7 cm) was frozen at the first sampling, all soil samples were collected from 7 to 17 cm throughout the experimental period. Soil samples were taken each month between February 15th and September 24th, 2021. Moreover, during manure applications, sampling frequency was increased, with soil samples taken just before and 24-48 h after manure application (Fig. 1). Soil samples were immediately placed in a cooling box before being transported to the laboratory for analysis of gross N turnover rates via the ^{15}N isotope pool dilution technique (Kirkham and Bartholomew 1954; Dannenmann et al. 2006). Each sample was carefully homogenized, sieved (2 mm) and coarse organic material and fine roots were removed. Subsequently, the soil samples were divided into four subsamples, 30 g for assessing background NH_4^+ and NO_3^- concentration (unlabelled), 30 g for gravimetric analysis of water content, and 200 g for labelling with $(^{15}NH_4)_2SO_4$ and 200 g with $K^{15}NO_3$ (Fig. S1). Spray flasks were used to label the soil samples homogeneously, with 6 ml of labelled solution sprayed onto the soil sample under constant remixing (Dannenmann et al. 2006). The ^{15}N-enriched soil samples were incubated for two hours (Fig. S1). Each soil sample was then separated into two subsamples of 80 g each and were put into plastic bottles. Without any further treatment one was considered time zero (t0) and the other soil subsample was covered with parafilm and immediately incubated in a small trench at original soil depth for 24 h and considered time one (t1). The
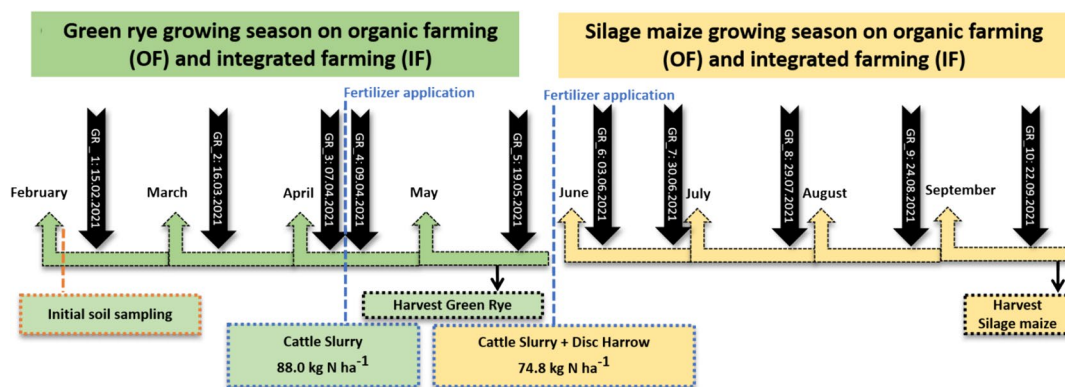

Fig. 1 Experimental and management activities on organic and integrated farming sites (OF, IF) in 2021. The green rye and silage maize growing periods are represented by green and yellow colour, respectively. Black arrows indicate the soil sampling and collection dates (GR_1 to GR_10) for gross N turnover. Fertilizer applications (blue) took place on April 8th, 2021, and May 31st, 2021. Seedbed for maize seedlings was prepared on May 31st, after manure incorporation. Weed control was carried out only in the silage maize growing season using a hoe on site OF (16/06/2021 & 29/06/2021) and herbicide ("Maister Power" and "Aspect", 2.00 and 1.50 l ha^{-1}) on IF (26/07/2021). Small black arrows indicate the harvest of green rye and silage maize in May and September, respectively

unlabelled and labelled soil samples ($t0$, $t1$) in the plastic bottles were extracted with 0.5 M potassium sulfate (K_2SO_4) using a soil:solution ratio of 1:2 during a 1 h shaking period at 150 rpm (Fig. S1).

Soil extract solutions were frozen at -18 °C until they were passed through the ^{15}N diffusion process in the laboratory. Following the method described in detail by Dannenmann et al. (2006) and Wang et al. (2016), acidified filter papers were used to trap NH_4^+ and subsequently NO_3^- through conversion to ammonium (Fig. S1). The filters were dried in a desiccator for 1 week, packed in tin capsules, and analyzed for ^{15}N enrichment using an elemental combustion analyser (Flash EA1112; Thermo, Bremen, Germany) coupled to an Isotope Ratio Mass Spectrometer (Delta Plus XP) as described by Vázquez et al. (2020). For the calculation of gross rates of N ammonification (a) and N nitrification (n), (Eq. 1) was used, while ammonium consumption (c_a), and nitrate consumption (c_n) were calculated according to (Eq. 2), referring to Kirkham and Bartholomew (1954).

$$a \quad \text{or} \quad n = \frac{M_{t0} - M_{t1}}{\Delta t} \frac{log\left(\frac{H_{t0}M_{t1}}{H_{t1}M_{t0}}\right)}{log\left(\frac{M_{t0}}{M_{t1}}\right)} \tag{1}$$

$$c_a \quad \text{or} \quad c_n = \frac{M_{t0} - M_{t1}}{\Delta t} \frac{log\left(\frac{H_{t0}}{H_{t1}}\right)}{log\left(\frac{M_{t0}}{M_{t1}}\right)} \tag{2}$$

M represents ^{14}N plus ^{15}N in NH_4^+ or NO_3^- concentration [mg N kg^{-1} dry soil], H represents ^{15}N in NH_4^+ or NO_3^- concentration [mg N kg^{-1} dry soil], subscripts t_0, and t_1 denote initial and post-incubation time, and Δt is time difference between t_0 and t_1 [days].

For the isotope pool dilution (IPD) a distinction between nutrient consumption and immobilization is necessary since ammonium consumption includes both microbial immobilization and nitrification (Davidson et al. 1991). For this reason, IPD ammonium immobilization was calculated according to (Eq. 3), described in Davidson et al. (1991), where $IPDi_a$ and $IPDi_n$ are immobilization rates of NH_4^+ and NO_3^- [mg N kg^{-1} dry soil d^{-1}], respectively; and n is the gross nitrification rate [mg N kg^{-1} dry soil d^{-1}]; c_a and c_n are the consumption rates of NH_4^+ and NO_3^-, respectively [mg N kg^{-1} dry soil d^{-1}], while NO_3^- immobilization (i_n) was considered to equal nitrate consumption.

$$IPDi_a = c_a - n \tag{3}$$

However, the addition of labelled ^{15}N NH_4^+ and NO_3^-, may stimulate immobilization so that consumption calculated by IPD may eventually overestimate actual immobilization rates. To account for this, the reformed difference method (RDM) was additionally used to calculate

immobilization rates ($RDMi_a$, $RDMi_n$, Hart et al. 1994a; Hu et al. 2019, Eq. 4 and 5).

$$RDMi_a = a - n - a_{net} \tag{4}$$

$$RDMi_n = n - n_{net} \tag{5}$$

$$a_{net} = \frac{[NH_4^+]_{t1} - [NH_4^+]_{t0}}{t} \tag{6}$$

where $RDMi_a$ and $RDMi_n$ are the reformed difference method immobilization rates of NH_4^+ and NO_3^-, respectively; a and n are the gross ammonification and nitrification rates, respectively, and a_{net} is the net ammonification rate [mg N kg^{-1} dry soil d^{-1}]. In analogy, n_{net} is calculated based on the NO_3^- concentrations. The rates $RDMi_a$ and $RDMi_n$ are, thus, independent of the nutrient consumption rates that may be influenced by substrate stimulation. Please note that the original difference method (Hart et al. 1994b) used additional unlabelled soil samples to study net ammonification and nitrification rates while net N turnover rates (a_{net}, n_{net}) for RDM were calculated from the changes in NH_4^+ and NO_3^- pool sizes between t_0 and t_1 (24 h) in the soil samples that were labelled with ^{15}N NO_3^- and ^{15}N NH_4^+ (Eq. 6).

To relate gross N turnover rates to amounts of fertilizer N application, they were converted to kg N ha^{-1} by considering soil bulk density, soil layer height and the representative time interval, for instance 2 weeks before and after sampling. Cumulative gross N turnover rates were calculated based on linear interpolation between measurement points.

Measurement of crop biomass and microbial biomass nitrogen

At each harvest, above- and below-ground plant biomass samples were collected for both green rye and silage maize. These samples were dried in an oven until they reached a constant dry weight. Subsequently, they were ground using a mixer mill (MM301). The ground samples were then packed into tin capsules and analyzed for TN content via elemental analysis coupled with mass spectrometry (Delta Plus XP), following the methodology described by Dannenmann et al. (2016).

Microbial biomass N (MBN) was determined on the date of green rye harvest using the chloroform fumigation-extraction method as summarized in Dannenmann et al. (2016). In brief, two sets of soil samples were taken, with one set being placed in a desiccator and fumigated with chloroform for 24 h to lyse microbial cells and release N. Subsequently, the fumigated and unfumigated soil samples were extracted with 0.5 M potassium sulfate (K_2SO_4) using a soil:solution ratio of 1:2 during a 1 h shaking period and total N in the extracted solution was measured

by DIMATOC (DIMATOC 2100, Analyses Technology GmbH, Nünningstr. 22–24, D-45141, Essen) to calculate the microbial biomass N using a conversion factor of 0.54.

Measurements of nitrous oxide emission

Soil N_2O emissions were determined using an automated gas sampling chamber system (Wolf et al. 2010). On each site, four subplots were established at the start of the experiment where a chamber frame was inserted 10 cm deep into the soil and on which one measurement chamber of $0.5 \times 0.5 \times 0.15$ m (length by width by height) was installed. These chambers were operated by a customized computer program controlling pneumatic actuators which opened and closed the chambers during the experiment. All four chambers installed on either site (OF or IF) were organized in one measuring block that closed and opened simultaneously. Closure time for each block was 48 min and each of the four chambers installed on a site was sampled four times for 3 min. Headspace air was continuously transferred to a gas analyser (GLA132 Series, ABB) located in a mobile laboratory placed on the border between the two sites. Chamber headspace air was analysed at a sampling frequency of approx. 1 Hz and soil N_2O fluxes were calculated from the linear concentration change over the chamber closure period. While chambers were installed between maize rows during silage maize cultivation, chambers contained plants during green rye cultivation. When plant height exceeded chamber height, chamber height was adjusted using extensions ($0.5 \times 0.5 \times 0.35$ m length by width by height) and the 0.15 m high chambers were fixed on top of these extensions. To calculate daily average N_2O emissions, the mean emission rates for the single chamber closure periods were averaged and the standard deviation was calculated. Cumulative N_2O emissions were calculated by conversion to kg N ha^{-1} considering the time between measurements and subsequent summation for the given period.

Measurements of weather and soil environmental conditions

Three temperature sensors (SE08012, Eckstein GmbH, 38,678 Clausthal-Zellerfeld, Germany) and three soil moisture sensors (SM150T moisture sensor, Delta-T Devices Ltd, Cambridge, United Kingdom) were installed in a subplot of the OF and IF sites at 5, 10, and 20 cm depth. Additionally, an air temperature sensor (NTC 10 K 1, Bessemerstraße 3, 21,339 Luneburg, Germany) was fixed to one of the subplots in the site at 1 m height. Precipitation data of the Gladbacherhof weather station near the site was used.

Data processing and statistical analysis

Gross N turnover rates were calculated using MS Excel software package (Microsoft Office 2019, Microsoft, Seattle, WA, USA). Figures were created using Origin 2020 (Origin Lab Corp., Northampton, MA, USA) and statistical analyses were conducted using SPSS (ver 27.0, IBM Crop., Armonk, NY, USA). The Shapiro Wilk test was used to test for normality of gross N turnover and mineral N data. If the Shapiro–Wilk test was passed, the Paired-sample t-test was used to test for significant differences. In the case of non-normally distributed data, the Wilcoxon signed-rank test was used. Statistical differences between N_2O emissions of the two sites were determined using the Wilcoxon rank-sum test. To characterize relations between soil gross N turnover and N_2O emissions as well as with soil environmental parameters, Pearson correlation analysis was performed using Origin 2020. Correlation analysis was based on the averages of N_2O emissions, soil temperature and soil moisture of the day at which gross N turnover rates were determined.

Results

Initial soil properties

Initial soil sampling revealed (Table 2) that pH in both 0–10 and 10–30 cm depth was significantly lower on OF (5.36 ± 0.06) than on IF (6.51 ± 0.19). With a layer height weighted average SOC content of $1.25 \pm 0.08\%$ (Table 2), IF exhibited a significantly higher SOC, and TN ($0.15 \pm 0.01\%$) content. Similarly, C:N ratio of IF was 10% higher than that of OF. Furthermore, OF had a slightly higher bulk density than IF. The soil texture analysis showed that both sites have silty loam texture, with nearly identical silt, sand, and clay contents (Table 2).

Crop biomass nitrogen and microbial biomass nitrogen

Significantly higher above-ground biomass nitrogen (AGB-N) during the green rye growing season was observed for site IF (Table 3). In contrast, AGB-N on OF exceeded that of IF in the silage maize growing season, but the differences were not statistically significant. There was no significant difference for the total sum of the whole growing season in AGB-N, and below ground biomass nitrogen (BGB-N) between sites OF and IF (Table 3), while microbial biomass N (MBN) was significantly higher for IF in both green rye and silage maize growing periods.

Table 3 Aboveground, belowground and microbial biomass N (AGB–N, BGB–N, MBN) on dates of harvest. Values are given in mean ± standard error (SE, $n = 4$)

Period	Site	AGB–N [kg ha^{-1}]	BGB–N [kg ha^{-1}]	MBN [kg ha^{-1}]
Green rye	OF	42.68 ± 3.08 [a]	12.32 ± 1.32	42.52 ± 3.22 [a]
	IF	82.77 ± 16.19 [b]	12.03 ± 3.45	73.23 ± 5.93 [b]
Silage maize	OF	151.57 ± 20.65	3.52 ± 0.78	77.24 ± 5.91 [a]
	IF	117.46 ± 3.29	2.07 ± 0.28	100.00 ± 9.22 [b]
Sum (whole growing season)	OF	194.25 ± 23.73	15.83 ± 2.10	119.76 ± 9.15 [a]
	IF	200.23 ± 19.48	14.10 ± 3.73	173.23 ± 15.14 [b]

Superscript letters depict statistically significant differences (t-test, $p < 0.05$) between organic farming (OF) and integrated framing (IF) sites

Soil environmental conditions

Volumetric soil water (SWC) content at both sites (OF, IF) ranged from 15 to 30% (Fig. 2a), corresponding to 30 to 65% water-filled pore space (WFPS), with no significant differences between sites. Soil temperature increased from 5 °C in March to 30 °C by mid-June (Fig. 2b) and dropped below 20 °C by September with a minor not significant difference

of 0.4 °C. Average air temperature was 7 °C during green rye and 18 °C during the silage maize growing season (Fig. 2b).

Soil ammonium (NH_4^+) and nitrate (NO_3^-) dynamics

In the green rye growing, NH_4^+ concentrations were slightly higher on site OF, showing significant differences on February 16th and April 7th. After slurry application on May

Fig. 2 Temporal course of environmental parameters and gross N turnover rates for sites organic farming (OF, blue colour) and integrated farming (IF, red colour). Panels show a) daily averaged volumetric soil water content (SWC) at 10 cm depth and daily precipitation (PPT, black bars), b) daily averaged soil temperature (ST) at 10 cm depth (OF in blue, IF in red) and air temperature (AT, black colour) at 1 m height, c) Mineral N content of ammonium (NH_4^+) and d) nitrate (NO_3^-) [mg N kg^{-1} dry soil d^{-1}], e) gross ammonification (*a*) and f) gross nitrification (*n*) at 7–17 cm depth [mg N kg^{-1} dry soil d^{-1}], and g) N_2O emission [μg N m^{-2} h^{-1}]. The solid blue and red lines with square symbols showed mean values of replicates and standard error. Dashed vertical black lines represent fertilization application events on April 8th, 2021, and May 31st, 2021. Green rye and silage maize growing period are represented by pale green and pale yellow background, respectively. Significant differences between OF and IF at a certain sampling date are marked by asterisks ($^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$)
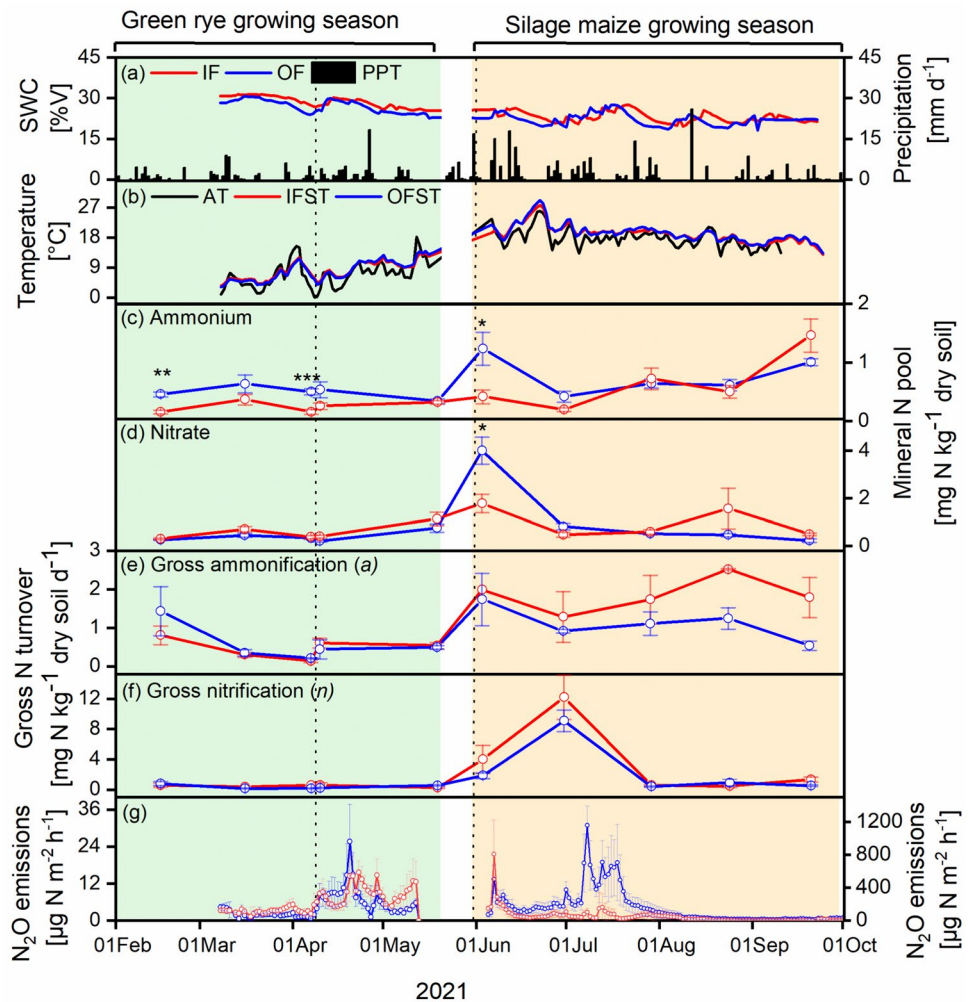
31st, NH_4^+ concentration increased significantly on OF, but not on IF (Fig. 2c). Average NH_4^+ concentrations on OF for green rye and silage maize growing seasons were 0.49 ± 0.04 and 0.78 ± 0.08 mg N kg^{-1} dry soil, and on IF were 0.25 ± 0.03 and 0.63 ± 0.11 mg N kg^{-1} dry soil, but differences were not significant.

The same pattern was observed for NO_3^- concentration, but concentrations were only significantly different on May 31st. During green rye growing, average NO_3^- concentration was 0.59 ± 0.09 mg N kg^{-1} dry soil and 0.40 ± 0.05 mg N kg^{-1} dry soil on IF and OF, respectively (Fig. 2d). Average NO_3^- concentration for silage maize growing season on IF and OF were 0.96 ± 0.19 and 1.24 ± 0.32 mg N kg^{-1} dry soil, respectively. Whole growing period averages were not significantly different (Fig. 2d).

Soil gross ammonification and nitrification rates

Soil gross ammonification rates (a) on OF and IF ranged from 0.21 to 1.44 mg N kg^{-1} dry soil d^{-1} and from 0.14 to 0.81 mg N kg^{-1} dry soil d^{-1} during green rye growing, with minimal temporal changes observed. During silage maize growing, a increased and ranged from 0.54 to 1.74 mg N kg^{-1} dry soil d^{-1} and from 1.29 to 2.52 mg N kg^{-1} dry soil d^{-1} (Fig. 2e) on OF and IF, respectively. During green rye growing, cumulative gross ammonification (a) rates were 67 and 58 kg N ha^{-1} (Fig. 4a and b) on OF and IF, respectively and increased to 162 and 276 kg N ha^{-1} during silage maize growing (Fig. 4c and d). Thus, gross mineral N production (a) in the 10 cm soil layer was in the range of plant N uptake (e.g., OF: $43 + 12$, IF: $83 + 12$ kg N ha^{-1} for green rye period; Table 3), so that mineral N production in the whole soil profile most likely by far exceeded plant N-uptake.

On average, a during green rye growing was 0.59 and 0.48 mg N kg^{-1} dry soil d^{-1} for OF and IF, respectively, with no significant difference (Table 4). During the silage maize growing, average a on IF was significantly higher (1.86 mg N kg^{-1} dry soil d^{-1}) than on OF (1.11 mg N kg^{-1} dry soil d^{-1}, Table 4). Silage maize growing cumulative ammonification was significantly (41%, Fig. 4c and d) higher on IF than on OF.

Soil gross nitrification rates (n) on OF ranged from 0.17 to 0.81 mg N kg^{-1} dry soil d^{-1} during green rye and from 0.43 to 9.11 mg N kg^{-1} dry soil d^{-1} during silage maize, while on IF it ranged from 0.26 to 0.61 mg N kg^{-1} dry soil and 0.48 to 12.22 mg N kg^{-1} dry soil d^{-1} respectively (Fig. 2f). Cumulative gross nitrification (n) rates for green rye were 45 and 56 kg N ha^{-1} (OF and IF, Fig. 4a and b) and increased to 426 and 586 kg N ha^{-1} during silage maize growing (OF and IF, Fig. 4c and d). Following the second slurry application in mid-May n, increased and remained elevated for the two following sampling dates.

On average, n for OF and IF were 0.39 and 0.49 mg N kg^{-1} dry soil d^{-1} during green rye growing, 2.57 and 3.73 mg N kg^{-1} dry soil d^{-1} during silage maize growing and 1.48 and 2.11 mg N kg^{-1} dry soil d^{-1} for the whole growing season, but IF rates were not significantly higher (Table 4).

Overall cumulative gross nitrification was several-fold higher than gross ammonification rates (a OF: 162, IF: 276 kg N ha^{-1}, n OF: 426, IF: 586 kg N ha^{-1}). Consequently, microbial mineral N production by far exceeded N uptake by plants (OF: $152 + 4$, IF: $117 + 2$ kg N ha^{-1}, Table 3). Cumulative gross nitrification peaked in July without corresponding rise in ammonification possibly due to heterotrophic nitrification. Excluding the data of July cumulative gross nitrification amounted to 138 and 196 kg N ha^{-1} for OF and IF, respectively, with total gross ammonification on OF and IF exceeding cumulative gross nitrification by 17% and 41%, respectively. The whole growing season cumulative values (Fig. 4e and f) are dominated by the silage maize growing season with minimal contribution of the green rye growing season.

Table 4 Average soil gross N turnover rates $\pm$ standard error (SE) of green rye, silage maize and whole growing season. Rows show gross ammonification (a), isotope pool dilution ammonium immobilization ($IPDi_a$) and reformed difference method ammonium immobilization ($RDMi_a$), gross nitrification (n), isotope pool dilution nitrate immobilization ($IPDi_n$) and reformed difference method nitrate immobilization ($RDMi_n$)

	Green rye (Feb-May) [mg N kg^{-1} dry soil d^{-1}]		Silage maize (Jun-Sep) [mg N kg^{-1} dry soil d^{-1}]		Whole growing season [mg N kg^{-1} dry soil d^{-1}]	
	OF	IF	OF	IF	OF	IF
a	0.59 ± 0.27	0.48 ± 0.11	1.11 ± 0.33 [a]	1.86 ± 0.44 [b]	0.85 ± 0.30	1.17 ± 0.27
$IPDi_a$	2.00 ± 0.47	1.73 ± 0.37	1.80 ± 0.77 [a]	4.46 ± 0.76 [b]	1.90 ± 0.62	3.10 ± 0.57
$RDMi_a$	0.87 ± 0.21	0.69 ± 0.21	1.16 ± 0.40	1.51 ± 0.31	1.01 ± 0.31	1.10 ± 0.26
n	0.39 ± 0.11	0.49 ± 0.24	2.57 ± 0.58	3.73 ± 1.29	1.48 ± 0.35	2.11 ± 0.77
$IPDi_n$	4.05 ± 1.28	2.08 ± 0.64	2.90 ± 0.86 [a]	5.57 ± 2.39 [b]	3.48 ± 1.07	3.83 ± 1.52
$RDMi_n$	0.69 ± 0.20	1.24 ± 0.36	2.13 ± 0.45	6.41 ± 2.63	1.41 ± 0.33	3.82 ± 1.49

Lower case letters (a, b) indicate significant difference between organic farming (OF) and integrated farming (IF) sites (paired t-test, $p < 0.05$)

Nitrous oxide emissions

At the start of the green rye season, N_2O emissions were below 8 µg N m^{-2} h^{-1} for both sites (Fig. 2g). After the slurry application N_2O emissions on OF and IF increased, with highest emissions of 25.6 and 15.6 µg N m^{-2} h^{-1}, respectively, throughout the green rye growing season. Except for nine days after fertilizer application, N_2O emissions on IF were marginally higher than OF (Fig. 2g). During silage maize growing season, N_2O emissions peaked after fertilizer application, reaching 809 µg N m^{-2} h^{-1} on OF and 502 µg N m^{-2} h^{-1} on IF. On June 30th, emission increased on both sites following moderate rainfall with the increase being more distinct on OF. However, in July, OF emissions permanently exceeded those of IF with the highest differences on July 7th, when OF emissions were 1160 µg N m^{-2} h^{-1} higher than those of IF (Fig. 2g), again associated with a period of moderate precipitation. Daily average N_2O emissions were significantly lower on OF during green rye cultivation and significantly higher during silage maize cultivation (Wilcoxon test, p < 0.05). Spatial variability of chambers fluxes was similar, with coefficient of variation averaging 41% on OF and 32% on IF for the whole growing period. Cumulative soil N_2O emissions were 4.36 kg N ha^{-1} (Fig. 4e) for OF and 1.45 kg N ha^{-1} for IF (Fig. 4f), accounting for 0.62% and 0.15% of the total sum of gross ammonification and nitrification, respectively. Cumulative N_2O emissions were not driven by a single chamber.

Soil gross microbial IPD and RDM immobilization of ammonium (NH_4^+) and nitrate (NO_3^-)

Soil gross ammonium and nitrate immobilization (i.e. i_a and i_n, respectively) were calculated using isotope pool dilution ($IPDi_a$ and $IPDi_n$) and the reformed difference method ($RDMi_a$ and $RDMi_n$; Fig. 3a-d, Table 4). Since gross rates are zero or positive by definition, $IPDi_a$ could not be calculated for sampling dates 2 and 7 (OF) and 6 and 7 (IF, Fig. 3a), and $RDMi_a$ could not be calculated for sampling date 7 (OF) and 6 and 7 (IF, Fig. 3b). $RDMi_n$ could not be calculated for sampling dates 5 and 10 (OF) and 5, 8 and 10 for IF (Fig. 3d).

Soil microbial $IPDi_a$ values ranged from 1 to 3 mg N kg^{-1} dry soil d^{-1} for site OF, and from 0 to 5 mg N kg^{-1} dry soil d^{-1} for site IF (Fig. 3a). Corresponding values for the $RDMi_a$ ranged from 0.10 to 2.18 mg N kg^{-1} dry soil d^{-1} for OF and from 0.28 to 2.17 mg N kg^{-1} dry soil d^{-1} for IF (Fig. 3b). As IPD gross immobilization overestimates due to the method-inherent addition of substrate, cumulative gross immobilization rates are only presented for the RDM method to better ensure compliance with cumulative gross ammonification and nitrification rates. Cumulative RDM gross immobilization of ammonium and nitrate were clearly smaller than IPD estimates.

Average $IPDi_n$ values ranged from 0.79 to 9 for OF and from 0.50 to 11 mg N kg^{-1} dry soil d^{-1} for IF, while $RDMi_n$ values ranged between 0.15 to 7 mg N kg^{-1} dry soil d^{-1} on OF and 0.64 to 10.49 mg N kg^{-1} dry soil d^{-1} on IF (Fig. 3c and d) for whole growing season. Average $IPDi_a$ was two and three times higher than the $RDMi_a$ values for OF and

Fig. 3 Temporal course of gross microbial N immobilization for sites IF (red) and OF (blue). Panels show (a) ammonium immobilization according to isotope pool dilution ($IPDi_a$), (b) ammonium immobilization according to reformed difference method ($RDMi_a$), (c) nitrate immobilization according to isotope pool dilution ($IPDi_n$) and (d) nitrate immobilization according to reformed difference method ($RDMi_n$). The whiskers show standard errors in mg N kg^{-1} dry soil d^{-1}. Dashed vertical black lines show the fertilizer application events on April 8th, 2021, and May 31st, 2021. Green rye and silage maize growing period are represented by pale green and pale yellow background, respectively
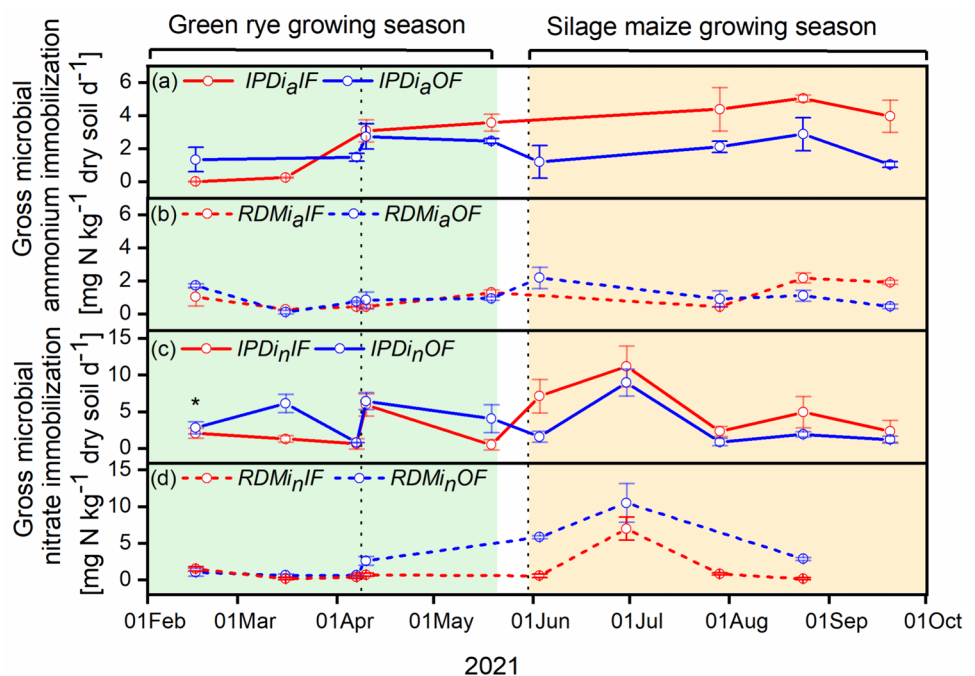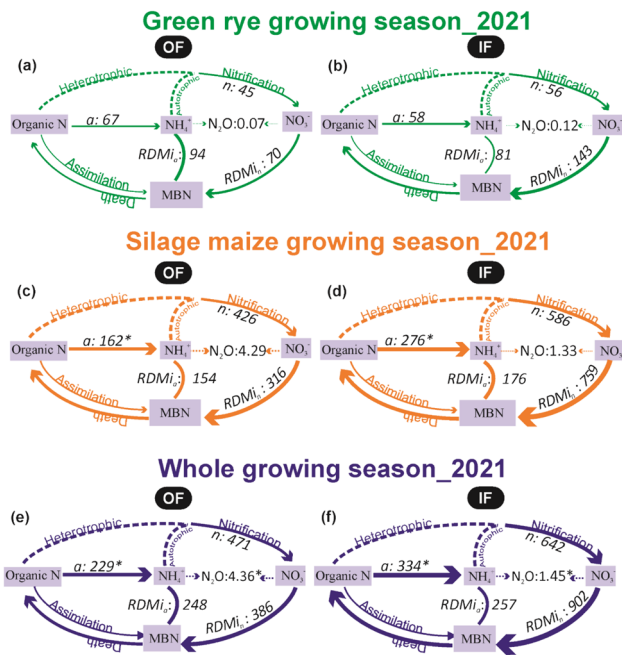
Fig. 4 Schematic flow chart of green rye (green arrows), silage maize (orange arrows), and whole growing season (purple arrows) cumulative gross N turnover rates [kg N ha^{-1}] for organic farming (OF) and integrated farming (IF) sites in the sampled 7–17 cm layer. Gross rates of ammonification, nitrification, NH_4^+ and NO_3^- immobilization (RDM), N_2O emission as well as microbial biomass N are indicated by a, n, $RDMi_a$, $RDMi_n$, and N_2O as well as MBN, respectively. Dashed lines emphasis that two substrates may affect the rate, while solid lines indicate single source pools. The total of apparently dominant autotrophic nitrification ($NH_4^+ \rightarrow NO_3^-$) and possibly heterotrophic nitrification (organic $N \rightarrow NO_3^-$) is referred to as gross nitrification. Similarly, NH_4^+ and NO_3^- can be substrates for N_2O production. Significant differences between OF and IF are marked by asterisks (paired t-test: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

IF respectively, and $IPDi_n$ was twice that of $RDMi_n$ on OF for whole growing season (Table 4). Significant differences between OF and IF ammonium and nitrate immobilization could only be detected for the IPD method during silage maize growing period (Table 4). The RDM cumulative NO_3^- immobilization rates exceeded cumulative nitrification rates on both sites during the green rye growing period, but not for the OF in the silage maize or whole growing season ($RDMi_n < n$; Fig. 4c and e). In contrast, during silage maize growing season, RDM ammonium immobilization was lower than cumulative gross ammonification on both sites ($RDMi_a < a$), but the sum of ammonium immobilization and nitrification exceeded gross ammonification ($a < n + RDMi_a$; Fig. 4c and d), which was also found for the whole growing season.

Correlation between gross N turnover rates with N$_2$O emission, and soil environmental parameters

Correlation analysis showed that for OF, gross ammonification was significantly positively correlated with gross nitrification and N_2O emission, while IPD ammonium immobilization as well as IPD nitrate immobilization did not show a correlation to gross ammonification and gross nitrification, respectively (Fig. 5). The opposite pattern applied to IF, where ammonification and nitrification were highly correlated to $IPDi_a$ and $IPDi_n$, respectively, but the correlation to N_2O emission was not significant. Gross ammonification (a), nitrification (n), and N_2O emission were significantly correlated with soil temperature for the OF site (Fig. 5). Similarly, a significant relationship for the IF site was observed between a, $IPDi_a$, and N_2O emissions with ST (Fig. 5). There was no significant relation to soil moisture for both sites.
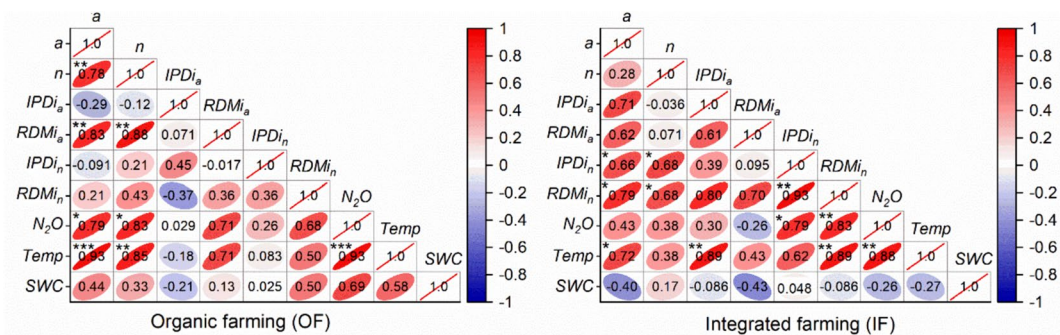

Fig. 5 Pearson correlation matrix for gross N turnover rates with N$_2$O emission and soil environmental parameters for organic and integrated farming sites (OF, IF). Quantities are gross ammonification (a), nitrification (n), soil microbial ammonium and nitrate immobilization according to isotope pool dilution ($IPDi_a$, $IPDi_n$), and reformed difference method ($RDMi_a$, $RDMi_n$), N$_2$O emissions, soil temperature (Temp) and soil water content (SWC). Numeric values represent the coefficient of determination, and asterisk the significance level (*: $p < 0.05$, **: $p < 0.001$, ***: $p < 0.001$)

Discussion

Dynamics and magnitude of gross N turnover rates

While gross ammonification rates ranged from 0.14 to 2.52 mg N kg^{-1} dry soil d^{-1} over the whole growing season, gross nitrification rates ranged from 0.17 to 12.22 mg N kg^{-1} dry soil d^{-1} for the same period. These results are consistent with other studies conducted on several agricultural systems that found that the majority of rates for gross ammonification and nitrification ranged from 0.20 to 3.0 mg N kg^{-1} dry soil d^{-1} and 0.40 to 10 mg N kg^{-1} dry soil d^{-1}, respectively (Booth et al. 2005; Cookson et al. 2006; Elrys et al. 2021). This shows that the body of studies conducted so far has fully characterized the seasonal variability for ammonification and nitrification observed in this study which was revealed through the high temporal resolution of measurements.

The same applies to IPD ammonium immobilization rates which ranged from 0.3 to 5 mg N kg^{-1} dry soil d^{-1} (average 2.3 mg N kg^{-1} dry soil d^{-1}) aligning with the range of 0.1 to 6 mg N kg^{-1} soil d^{-1} reported for soils with C contents of less than 2% (Booth et al. 2005) and the average value for croplands (~ 3 mg N kg^{-1} dry soil d^{-1}) in a recent review on IPD immobilization rates (Elrys et al. 2021; He et al. 2021; Wang et al. 2023a). In contrast, nitrate immobilization on OF and IF ranged from 0.5 to 11 mg N kg^{-1} dry soil d^{-1} (average 3.7 mg N kg^{-1} dry soil d^{-1}) and showed higher maximum values compared to the 0.04 to 2 mg N kg^{-1} dry soil d^{-1} reported for the class of soils with less than 2% C by Booth et al. (2005) and the reported values of less than 1.5 mg N kg^{-1} dry soil d^{-1} for cropland by Elrys et al. (2021). The same pattern was identified for RDM immobilization rates by comparing measured values of this study with the study of Hu et al. (2019). Since the peak values of NO_3^- immobilization occurred in the absence of simultaneously increasing nitrification, a boost of heterotrophic nitrification may have caused the high NO_3^- immobilization. Such a boost of heterotrophic nitrification could result from organic N inputs through manure application, which was not part of the management of Hu et al. (2019), and which might have been missed in the observations of the cited review articles (Booth et al. 2005; Elrys et al. 2021). This indicates that so far, the range of immobilization rates occurring in agricultural systems receiving organic fertilizers has not been fully characterized, highlighting the need for further studies covering whole growing seasons and particularly times after manure application.

In the context of the magnitude of gross N turnover rates, heterotrophic nitrification is a major complication for the RDM method since the nitrified organic material doesn't pass the NH_4^+ pool, so that heterotrophic nitrification is not reflected in ammonification measurements. This violates the mass conservation equation underlying the RDM method, which is especially problematic for NH_4^+ immobilization since overestimated nitrification can turn the immobilization rate negative which is per definition not possible and the values have to be discarded. Since we cannot exclude a bias of RDM immobilization values due to heterotrophic nitrification, we will focus on IPD immobilization rates in the discussion of this manuscript.

Regarding the determination of immobilization rates using the IPD method, average microbial IPD NH_4^+ and NO_3^- immobilization rates by far exceed the gross ammonification and nitrification rates for the whole growing season (Table 4). Since IPD immobilization is based on the consumption rates of the labelled species that are applied to the soil samples, they are inherently stimulated by the ^{15}N NH_4^+ and ^{15}N NO_{3-} additions and, hence, an overestimate. While this is definitely problematic for studying actual immobilization rates, substrate stimulation occurs in agricultural systems through fertilizer input. Thus, IPD immobilization rates can be rather interpreted as potential immobilization rates, and direct site comparison can be used to identify sites with higher potential to balance excess N by the internal immobilization and remineralization cycle (Kreutzer et al. 2009). Sites with this potential may be less vulnerable to N loss, especially through denitrification. Consequently, the significantly higher IPD NH_4^+ and NO_{3-} immobilization rates observed during the silage maize growing season indicate a lower vulnerability towards N loss for the IF site. This effect may have been concealed due to the low temperatures and, thus, generally lower rates during green rye cultivation. Nevertheless, the IPD method not necessarily overestimates immobilization rates since Hart et al. (1994b) reported that in forest soil the immobilization rates estimated by IPD and the difference method were in close agreement, suggesting that low-level ^{15}N inputs [0.6–1.2 mg N kg^{-1} soil] may not constantly stimulate ambient immobilization.

Impact of management history and soil environmental parameters on soil gross N turnover rates

The initial soil sampling revealed differences in soil properties (Table 1) due to different long-term management histories with microbial biomass N being significantly higher on IF compared to OF (Table 4). Though conventional management typically results in lower SOC accumulation and microbial biomass compared to organic management (Gattinger et al. 2012; Lori et al. 2017), C and N content was 37% and 25% higher on IF compared to OF. Organic fertilizers and a combination of organic and synthetic fertilizers positively affect SOC accumulation (Hai et al. 2010; Dai

et al. 2017) since higher fertilizer C input balances C outputs through ecosystem respiration and harvest. Similarly, increased biomass production contributes to SOC accumulation through greater root growth and associated belowground C input through root exudates and decaying roots. Consequently, more frequent and higher C (and N) additions through residue return and organic fertilizers (cattle slurry, sewage sludge) on IF compared to OF, as well as legume biomass removal on OF may partially explain the SOC differences. Another reason for higher SOC content may be the higher biomass production in conventional farming systems (Seufert et al. 2012). We assume that during the period of different preceding management, i.e., 2012–2020 (Table 1), higher fertilizer N input boosted biomass production on IF, which aligns with other studies showing that long-term application of organic manure combined with mineral fertilization could increase crop yield, soil C and soil N content (Gai et al. 2018; Böhm et al. 2020). In this context, the higher gross ammonification, nitrification and significantly higher IPD NO_3^- immobilization rates for IF were likely due to the higher SOC, TN content, and microbial biomass which in turn positively influences microbial N turnover (Nieder and Benbi 2008; Dai et al. 2017; Elrys et al. 2021).

Gross N ammonification and nitrification rates for OF and IF demonstrated seasonal variation during the growing season (Fig. 2e, f). Though numerous studies conducted in various ecosystems found that increasing temperature lead to increased gross and net N mineralization (Hart 2006; Larsen et al. 2011; Björsne et al. 2014) and had a slight effect on nitrification (Auyeung et al. 2013), only the relation of gross ammonification with soil temperature was significant in our study. In contrast, a significant correlation of gross N turnover and soil water content could not be shown in our study. However, factors like fertilization, soil preparation, and their interactions may dominate the magnitude of N turnover rates, thereby overwriting temperature and moisture effects. For instance, tillage promotes soil physical degradation, breakdown of soil organic matter and microbial biomass, increases SOC by incorporation of crop residues, and enhances N mineralization activity (Haddaway et al. 2017; Jha et al. 2022). Similarly, readily accessible C and N in organic manure boost microbial biomass and activity, affecting mineralization and nitrification rates (Mallory and Griffin 2007; Müller et al. 2011).

Implications of gross N turnover rates and historic management for N₂O emissions

Nitrous oxide emissions peaked on both OF and IF directly after fertilizer applications, with the highest values in April being much smaller than those in June and small differences between sites. However, distinctly higher N₂O emissions from OF were observed approximately one month after fertilizer application, coinciding with the highest nitrification rates on both sites. Cumulative N₂O emissions amounted to 4.29 kg N ha^{-1} on OF and 1.45 kg N ha^{-1} on IF (Fig. 4e, f). While other studies also noticed that long-term organic farming systems showed increased N₂O emissions compared to conventional farming systems, when fertilized identically (Krause et al. 2017), the cumulative emissions of our study are within the range of 1.9 to 4.8 kg N ha^{-1} reported for conventional silage maize systems (Weller et al. 2019; Maier et al. 2022). While research on nitrous oxide emissions is extensive with regard to total surface emissions, knowledge on the distribution of N₂O production within the soil profile is scarce. Recent research showed that especially in annual crops, more than 50% of N₂O emissions measured at the surface were generated in the top 20 cm of soil, confirming that N turnover at 7–17 cm depth significantly contributed to surface N₂O emission (Hosen et al. 2000; Shcherbak and Robertson 2019).

The lack of strong N₂O emission on site IF may be due to the combination of several reasons. On OF, the correlation between gross ammonification and nitrification is significant, while gross ammonification and IPD ammonium immobilization, and gross nitrification and IPD nitrate immobilization are uncorrelated (Fig. 5). On IF, the correlation between gross ammonification and nitrification is not significant, while gross ammonification and IPD ammonium immobilization, and gross nitrification and IPD nitrate immobilization are significantly correlated (Fig. 5). This and the significantly higher IPD immobilization rate on IF indicate that the internal immobilization-remineralization cycle (Paul and Beauchamp 1995; Kreutzer et al. 2009; Douardo-Neto et al. 2010) may balance excess NH_4^+ for nitrification and excess NO_3^- available for denitrification and N₂O production. This is supported by the significant correlation of gross nitrification and N₂O emissions for OF (insignificant for IF) and the ratio of N₂O emissions to cumulative nitrification, which amounted to 0.92% for OF and only 0.23% for IF. In this context, it is noteworthy that we did not observe significantly lower NO_3^- concentrations on IF despite the higher IPD NO_3^- immobilization. This may be due to the fact that the IPD rate rather represents an immobilisation potential than the actual rate which may scale with nitrate availability. In addition, poor correlation between gross N turnover rates and the size of mineral N pools as it occurred in this study has been observed before (Hart et al. 1994a).

Furthermore, the lower SOC content observed on OF may have affected soil pH. In a study comprising 34 soil types, a significant positive linear relationship was found between soil buffering capacity (the ability of soil to resist changes in pH when acids or bases are added) and C content. For this reason, the greater abundance of functional molecular groups in soil organic matter may have increased the buffering capacity of IF (Curtin and

Trolove 2013), resulting in a higher pH. In addition, repeated alfalfa cultivation in two consecutive years on OF may have contributed to the lower pH of OF since Uzoh et al. (2019) and Wang et al. (2020a) reported a significant decrease in soil pH during legume cultivation in a velvet bean-maize crop rotation due to organic acid release from roots during the symbiotic N_2 fixation. Soil pH and N_2O emissions are related since N_2O is converted to N_2 by the enzyme N_2O reductase. The synthesis of this enzyme is inhibited by low pH, which consequently promotes N_2O production and emission (Russenes et al. 2016; Ouerghi et al. 2023). Similarly, Wu et al. (2024) reported that in acidic soils (pH 5.2 and 4.2), manure addition increased soil nutrients and bacterial activity, which led to higher N_2O emissions compared to synthetic fertilizer.

In addition, less frequent tillage due to differences in crop rotation and low SOC on site OF is likely to be the reason for the higher bulk density (1.44 ± 0.02, Table 1) compared to IF, with higher bulk density and thus lower aeration contributing to higher N_2O emission (Krauss et al. 2017).

Finally, alfalfa preceded green rye growing and silage maize growing on the OF site. Legumes have been reported to lead to increased N_2O emissions in the subsequent crop-growing period, due to the mineralization of legume residues (Drury et al. 2008; Adviento-Borbe et al. 2010; Skinner et al. 2014; Saha et al. 2021; Franco-Luesma et al. 2022). Though the measurements show a similar time course of mineralization and nitrification, these turnover rates were not measured at the peak N_2O emissions, so that differences in the mineralization of legume residues cannot be specifically excluded.

Conclusion

A direct comparison of soil gross N turnover rates of an organic farming site and an integrated farming site revealed that gross ammonification, ammonium immobilization and nitrate immobilization were significantly increased on the integrated farming site during the silage maize growing period. The higher soil gross N turnover rates were most likely due to higher SOC content and microbial biomass that resulted from the long-term different management history. Increased immobilization rates were associated with lower N_2O emission during periods of high nitrification, indicating that the combination of mineral and organic fertilizers strategy on IF farming reduced the vulnerability to N losses. The high temporal resolution of gross N turnover measurements revealed that so far, the range of immobilization rates of agricultural systems receiving organic fertilizers has not been fully characterized, so that future studies covering whole growing seasons and particularly times after manure application are pertinent.

Declarations

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