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Microbial inoculant has little effect on greenhouse gas emissions following cover crop incorporation

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ABSTRACT

A net negative emissions technology is the transformation of CO_2 and its storage in agricultural soils in form of soil organic carbon (SOC). One possibility to increase SOC stocks in agriculture is to grow and incorporate cover crops in the upper soil layer. However, incorporation of fresh plant material can also increase N_2O emissions and thereby reduce the overall greenhouse gas mitigation effect. While the effect of removing plant material is relatively well understood, the effect of different incorporation methods and the inoculation of cover crops with microbial inoculant (MI) is still poorly known. To investigate these effects, we conducted an incubation study and a field trial where a grass-clover cover crop was followed by maize. We measured greenhouse gas emissions (N_2O, CO_2), soil parameters (Nmin, DOC, soil moisture and temperature), soil organic carbon (SOC) stocks and maize yield. In the four weeks following cover crop incorporation, shallow rotary tillage induced 30 % higher CO_2 emissions than ploughing and removal of cover crop biomass resulted in significantly lower N_2O and CO_2 emissions as if it was mulched and inoculated with MI. Regarding the whole season, removal of aboveground cover crop biomass reduced N_2O field emissions in tendency by 21 %, whereby the trend in N_2O reduction by adding MI in the field was less pronounced. Total N_2O emissions did not differ between tillage implements used for incorporation. SOC stocks did not change within 0–20 cm within a year. Maize yield was 23 % higher with ploughing than rotary tillage. Overall, the addition of MI during cover crop incorporation might improve the greenhouse gas balance, but potential effects are superimposed too strongly by other management and meteorological factors. Therefore, claims that MI are an option to mitigate greenhouse gas emissions from agriculture remain weakly substantiated.

1. Introduction

To fight global warming, a drastic reduction of greenhouse gas emissions and net negative emissions technologies are required (Rogelj et al., 2015; Seneviratne et al., 2018). One of the technologies discussed is the transformation of CO_2 and its storage in agricultural soils in form of soil organic carbon (SOC). The international initiative "4 per 1000" suggests a set of measures that should help increasing SOC stocks worldwide at an annual rate of 4 ‰. One of the possibilities to increase SOC stocks in agriculture is to grow and incorporate cover crops in the upper soil layer (Guenet et al., 2021).

However, cover crops also enhance the risk of substantial N_2O emissions, which may offset the CO_2 mitigation potential (Basche et al., 2014). Especially the incorporation of large amounts of cover crop biomass transfers easily degradable carbon and nitrogen into the soil at once. Thereby the amount of biomass, its C:N ratio, stage of physiological maturity and frost tolerance (Abalos et al., 2022b; Böldt et al., 2024) as well as the type of tillage by which it is incorporated into the soil have an effect on resulting N_2O emissions (Abalos et al., 2022a; Hansen et al., 2019). While Abalos et al. (2022a) found lower N_2O emissions following shallow incorporation, Hansen et al. (2019) concluded in their review that placing cover crops with rotating machines close to the soil surface may induce larger N_2O emissions. Removing aboveground parts of cover crops before soil tillage may partly mitigate this trade-off as its removal can reduce up to 40 % of the subsequent N_2O emissions (Abalos et al., 2022a).

In Europe, the plough is still commonly used for soil tillage (Eurostat, 2016). Yet, conservation tillage has increasingly gained in importance in terms of soil quality including positive effects on SOC stocks in top-soils (Haddaway et al., 2017; Krauss et al., 2022) but also the risk to increase

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N_2O emissions (Abdalla et al., 2013). “Regenerative” farmers in Germany, Austria and Switzerland often use a combination of shallow cover crop incorporation in parallel with an application of microbial inoculants (MI) (e.g. “Rottelenker”). Thereby, cover crops are shallowly incorporated into the topsoil sometimes without previous mulching and often with power-driven rotary tillers. Microbial inoculants are directly sprayed into the soil – cover crop mixture during incorporation. ”Rottelenker” is a mixture of effective microorganisms, molasses and fermented herbs and is claimed to positively affect cover crop decomposition before the main crop is sown. The intention is to minimize nutrient loss from the soil-plant system, to support the growth of the main crop and to increase SOC sequestration (Regenerativ Schweiz, 2023). The combination of shallow cover crop incorporation combined with MI have, to our knowledge, not yet been investigated scientifically in regard to SOC sequestration and greenhouse gas mitigation potential.

The few published studies with effective microorganisms showed no effect on SOC (Breza-Boruta and Bauza-Kaszewska, 2023; C. Hu et al., 2018). Microbial inoculants have already been investigated as a measure to improve rice straw decomposition in paddy rice or wheat-rice systems in Asia. Overall, the addition of microbial inoculants to straw showed either no effect on N_2O and CH_4 emissions or a reduction in N_2O and an increase in CH_4 emissions after incorporation (Hao et al., 2022; Liu et al., 2015, 2019a; Ma et al., 2019; Wang et al., 2020). Yet, straw is a mature material with a higher C:N ratio as most cover crops and paddy rice systems are a temporally flooded environment. Results can thus not directly be compared to the European arable setting. The low amount of studies regarding the effect of microbial inoculants on greenhouse gas emissions have also surprised Abdalla et al. (2013), who conclude in their review that field measurements in varying pedoclimatic conditions are crucial to correctly evaluate their effect.

To investigate the increasingly popular practice, we set up a lab and a factorial field study. Our hypotheses based on the claims of the "regenerative agriculture" movement and available literature were that (I) total N_2O emissions are reduced and SOC stocks enhanced by the application of MI in combination with the shallow incorporation of a cover crop (II) a larger reduction in N_2O emission is achieved by the removal of aboveground parts of cover crops, without compromising SOC stocks.

2. Materials and methods

2.1. Incubation study

To initially assess potential effects of MI on N_2O and soil N dynamics following the incorporation of a cover crop under controlled conditions, we run an incubation study with three treatments: (I) incorporation of grass-clover (m), (II) incorporation of grass-clover inoculated with MI (m+), and (III) a control (soil) without additions (c). The process is illustrated in Figure S2(Supplement).

We took soil from the first 5 cm of a vegetable field in Möhlin (47°32’52.1"N 7°51’18.1"E, pH = 6.3; C/N ratio = 9.1; 16 % clay, 73 % silt, 16 % sand) in August 2020. The soil was sieved to 5 mm aggregates and air-dried at room temperature. As MI, we chose fluid “Rottelenker” (EM Schweiz, CH) with a microbial composition containing *Lactabacillus casei* ($2.1*10^4$ KBE ml^{-1}), *Lactabacillus plantarum* ($2.3*10^5$ KBE ml^{-1}), *Saccharomyces cerevsae* ($3.1*10^4$ KBE ml^{-1}) and *Rhodopseudomonas palustris* ($1.6*10^4$ KBE ml^{-1}). One day before the incubation started, we filled fifty-four 250 ml DURAN wide neck glass bottles (Schott AG, Mainz, Germany) with 100 g dried soil each. To start the incubation, we harvested red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) from a grass-clover ley, homogenized the plant biomass and split it into two equal parts. For treatment m, we shredded one part with a blender (Steel Line, König, DE), homogenized it manually and mixed an aliquot of 9 g in each bottle with the soil it contained. This amount equals the incorporation of 30 t ha^{-1} fresh clover in the field. For treatment m+, we took the second part of the plant biomass and repeated the procedure, yet treated the clover biomass with an equivalent of 150 L ha^{-1} MI, using an aerosol sprayer (Foxy Plus 360°, Birchmeier, CH), before shredding. In all glass bottles, including the control treatment with no plant biomass addition (c), we compacted the soil blend to a bulk density of 1.25 g cm^{-3}. Finally, we added water until the water-filled pore space (WFPS) reached 65 %. Bottles were incubated at 20°C in an incubator and covered with perforated aluminum foil to protect against water loss. We distinguish between two sets. One set was used to determine gas fluxes (set 1) and another to destructively subsample soil for the analysis of N dynamics (set 2). We measured N_2O and CO_2 over 40 days by directly placing the same incubation bottles of set 1 onto the autosampler of a gas chromatograph (7890 A; Agilent Technologies, Santa Clara, CA; N_2O detection by ECD and CO_2 by an FID). Throughout the first three days, we measured gases every three hours, then every morning from day 3 to day 24 and thereafter once every third day until day 40. On day 0, 0.4, 2, 7, 14, 21 and 40, we determined the ammonium and nitrate concentration of three replicates of each treatment by destructively sampling set 2. We took an aliquot of 30 g soil-plant mixture and extracted it with 120 ml of 0.01 M $CaCl_2$ solution. We analyzed the extracted solution for mineral nitrogen (nitrate and ammonia) with an ion chromatograph (IC 940, Metrohm, CH).

2.2. Field study

2.2.1. Site conditions, field trial design and management

The field trial was set up in Frick (Switzerland, 47°30’42.6"N 8°01’23.5"E, 350 m altitude) in autumn 2021. The soil was a Vertic Cambisol (WRB, 2015). The calcareous clay loam (45 % clay, 27 % silt, 28 % sand) exhibited considerable swelling/shrinking properties (Fontana et al., 2015) and had a mean pH (H_2O) of 7.1 in the upper 0.1 m. The mean temperature in 2022 was 11.8°C and the mean annual precipitation was 878 mm. The preceding crop was winter wheat.

In a randomized block design with four replicates, we tested six treatments: removing the aboveground biomass of a cover crop before rotary tilling (Rr) or ploughing (Pr), mulching the cover crop before rotary tilling (Rm) or ploughing (Pm), and treating the cover crop in addition with MI during mulching before rotary tilling (Rm+) or ploughing (Pm+). Rotary tilled (R) plots were 2.8 m wide and ploughed (P) plots 4.8 m wide. Plot length was between 14 and 28 m.

A cover crop mixture (Lolinca Bio, UFA, CH) consisting of *Trifolium pratense L.*, *Trifolium incarnatum L.*, *Lolium multiflorum Lam.* and *Lolium multiflorum Lam var. westerwoldicum Mansh.* was sown on 25th September 2021 in all plots with a seeding density of 0.325 g m^{-2}. The seedbed preparation was done with a rototiller (5 cm depth). On 3rd March 2022, cattle slurry of the farm was applied into the cover crop with a drag hose at a rate of 40 m^3 ha^{-1} as a pre-fertilizer application for maize (1.8 % dry matter content, 43.7 g dry matter kg^{-1} Kjehldahl-N, N input 31.6 kg N ha^{-1}). Farm operations differentiating treatments are illustrated in Figure S3(Supplement). In treatments Rr and Pr, we mowed and removed the aboveground biomass on 19th April 2022. The next day, the aboveground biomass was mulched in Rm and Pm (SAX 1600, Flac, IT). In Rm+ and Pm+ we applied a total of 75 L ha^{-1} MI on the cover crop before mulching and another 75 L ha^{-1} before incorporation. For accurate application, we used a power backpack sprayer (433, Solo Kleinmotoren GmBH, DE). Directly after mulching, the P plots were first ploughed (Vari-Master 121, Kuhn, FR) to 20 cm and the soil flattened with a horizontally working rotary harrow thereafter (MX300, Remac, IT). The R plots were superficially tilled to a maximum of 5 cm with a power driven vertically working rotary tiller (VES 285, Cutter, AU; 540 U min^{-1}, 5–6 km h^{-1}). The process from mulching to incorporation was done within a maximum of 30 minutes. On average 8.8 t DM ha^{-1} cover crop (above and belowground biomass) were incorporated in the mulched treatments and 6.3 t DM ha^{-1} belowground biomass in the treatments Rr and Pr (Table 1). We tilled the R plots again with the same rotary tiller to 6 cm on 17th May 2022 and one day later worked all plots with a rotary harrow for seedbed preparation and sowing of silage maize (Hybrid Maize, KWS) with a row distance of

Table 1
Cover crop biomass aboveground (shoot) and belowground (root), its C:N ratio and the nitrogen input by the cover crop per hectare (further details on biomass measurements in 2.2.5).

	Biomass (t DM ha^{-1})	C:N ratio	Cover crop N input (kg N ha^{-1})
Shoot	2.5 (±0.3)	25.5	40.0
Root	6.3 (±1.9)	22.3	86.7
Total	8.8		126.7

50 cm and a seeding depth of 5–6 cm in all plots. On 15th June 2022, we distributed 500 kg ha^{-1} organic manure pellets (60 kg N ha^{-1}; N-Bio 12 %, Landor, CH) into the maize rows by hand, before hoeing the entire field for weed control (KPP-F 6×50, Schmotzer, DE). Maize harvesting took already place on 24th August 2022 as the maize matured early due to extremely dry weather conditions in July and August 2022.

2.2.2. Greenhouse gas monitoring

We measured nitrous oxide (N_2O) and carbon dioxide (CO_2) with closed static chambers. Measurements started shortly before slurry application into the cover crop in March 2022 with one chamber base ring (30 cm diameter, 15 cm height) per plot installed into the soil (10 cm depth), in the middle of each plot and in 3 m distance to plot margins. Following slurry application, we measured trace gas fluxes four times within seven days and weekly or bi-weekly thereafter until cover crop termination. After cover crop incorporation in April 2022, we installed two rings per plot (n = 8 per treatment) at a distance of seven meters, avoiding tractor lanes. Intensive sampling, daily to three times a week, continued until maize sowing. For seedbed preparation and sowing, we removed the base rings for two days and then placed them between the maize rows, not in the tractor lane and as close as possible to their previous location. For fertilization and hoeing, we removed the base rings for one day and installed them in the same place afterwards.

During trace gas sampling between 9 and 12 am, we placed a vented PVC chamber (30 cm diameter; 12 cm height) onto each base ring and extracted four gas samples over the course of 40–50 minutes. Loggers (IBS-TH2, Inkbird) tracked the air temperature inside the chambers. We took 20 ml of air with a syringe and injected the sample into an evacuated 12 ml Exetainer (Labco Ltd. UK) for analysis with the same setup as used in the incubation study. Fluxes were calculated with the *gasfluxes* R-script elaborated by Hüppi et al. (2018). The chambers we used have been described by Flessa et al. (1995), and the gas sampling process, analysis and flux calculation by Krauss et al. (2017).

2.2.3. Soil sampling and analysis

Ancillary soil samples were taken with a soil auger (1 cm diameter) to a depth of 20 cm with six pooled replicates per plot. The soil was stored at 4°C until processing within max. 24 hours. We sieved the soil samples to 5 mm aggregates, determined the gravimetric water content and extracted 30 g of soil with 120 ml 0.01 M $CaCl_2$ solution. Extracts were frozen until analysis. We analyzed mineral nitrogen (nitrate and ammonia) with an ion chromatograph (IC 940, Metrohm, CH) and DOC with a TOC analyzer (TOC-L, Shimadzu, JPN) from the same extracts at the same time.

2.2.4. Soil sampling for soil organic carbon stocks

For assessing changes in soil organic carbon (SOC) stocks, we sampled the soil twice, after harvesting wheat on 18th August 2021 and after the maize harvest on 24th August 2022. We collected soil cylinders with special augers (d = 5 cm, Sample ring kit C, Eijkelkamp, NL) from the soil layers 0–7 cm and 7–20 cm. We defined a GPS tracked position in each plot from which we took three individual pseudo replicates within a radius of 1 m. For each cylinder, we determined dry bulk density gravimetrically and SOC content with a combustion analyzer (RC612, Leco, USA), which measured SOC (at 550°C) and soil inorganic carbon (at 1000°C). Because incorporation treatments altered bulk densities between 2021 and 2022, we applied the equivalent soil mass (ESM) approach for SOC stock determination on the basis of published equations of von Haden et al. (2020) and Leifeld et al. (2011). Firstly, we calculated the bulk density of fine soil (FD, g cm^{-3}) as follows:

$$FD = M_f / (V_t - (M_c / 2.4))$$

where M_f is the dry fine soil mass (g, <2 mm), V_t is the total volume of the sample (cm^3) and M_c is the mass of the coarse fragments (g), which is divided by the density factor of 2.4 g cm^{-3} (Schwab and Gubler, 2016).

Next, we calculated the cumulative soil mass (M_{cum}; Mg ha^{-1}) and cumulative soil organic carbon mass (SOC_{cum}; Mg ha^{-1}) for the soil layers of 0–7 and 0–20 cm as follows:

$$M_{cum} = FD \text{ x } h \text{ x } 100$$

$$SOC_{cum} = M_{cum} \text{ x } SOC / 100$$

with h as the layer thickness (cm) and SOC as the organic carbon content (%) of the respective layer. The cumulated SOC masses per layer (x-axis) were plotted against the respective soil masses (y-axis) that were cumulated with soil depth. From three data points per soil layer and plot, we extrapolated a cubic spline regression based on the R script provided by Krauss et al. (2022). As reference soil masses, we took the mean M_s per soil layer across all plots in 2021. Accordingly, we calculated SOC masses per plot and soil layer using the respective cubic spline regression.

2.2.5. Biomass yield and quality

To determine the amount of cover crop biomass incorporated in the different treatments, we sampled the above- and belowground biomass. Per plot, we took three replicate samples of aboveground grass-clover biomass from 30 ×30 cm subplots and dried them at 60°C for 24 hours. Belowground biomass was only roughly estimated by sampling roots at five representative locations across the field trial to a soil depth of 15 cm with a root sampler (d = 8 cm, Root auger, Eijkelkamp, NL). The root soil cores were stored at 4°C until processing. Soil cores were crushed into smaller pieces, soaked and washed through a 0.5 mm sieve and roots dried at 60°C for 24 hours. The dried above- and belowground cover crop samples were milled (SM100; Retsch, DE) and then analyzed for C and N with an elemental analyzer (CN628; Leco, USA).

Maize plants were harvested manually by cutting a total of six meters of maize rows in close proximity to the flux chambers. We determined the total fresh plant in the field (60K5A, Kern, DE), shredded plants and cobs separately (S-B5 (3,8 kW), Samix) and dried the representatively sampled subsamples at 60°C for 24 hours. Subsamples were analyzed for C and N with an elemental analyzer (CN628; Leco, USA).

2.2.6. Data processing and statistics

We did all data processing and statistics in R (R Core Team, 2020). We determine differences in the incubation study with a t-test between m and m+. In the field study, we took first the arithmetic means of the two pseudo-replicated chambers per plot before running the analysis of the gas data. To determine differences in gas flux, soil and plant data, the incorporation (P, R) and cover crop treatments (r, m, m+) were set as fixed factors and blocks as random effect in a variance analysis (ANOVA). For the SOC stock changes, we assessed changes between 2021 and 2022 with a time series ANOVA using the "nlme" package (Pinheiro et al., 2020). Temporal dependencies were considered in the correlation term.

3. Results

3.1. Incubation study

During a 40-day incubation, only little mineralization occurred in the control treatment without any addition (c), as indicated by slowly increasing nitrate concentrations that did not affect soil gas fluxes (Fig. 1). In contrast, addition of chopped clover biomass introduced dissolved organic carbon (DOC). DOC concentrations decreased faster in m than m+ showing a significant difference of 63.3 mg DOC kg^{-1} at day 2. Ammonium and nitrate contents, together with CO_2 fluxes, indicated a high microbial activity within the first 20 days in both m and m+. At day 14, there was a trend towards lower NO_3^- (11.8 mg N kg^{-1}; p=0.78) and higher NH_4^+ contents (25.4 mg kg^{-1}; p=0.22) in m+, as compared with m. In m and m+ we detected three N_2O peaks. Short peaks occurred after nine hours, two days, and a longer lasting peak appeared with a maximum on day 14 and elevated background fluxes thereafter in m and m+ until the end of the incubation. While there was no significant difference during the first peak, N_2O fluxes were lower in m+ than in m during the subsequent two peaks. Cumulative N_2O emissions over 40 days were 16.1 (±5.72) mg N_2O-N kg^{-1} soil in m and 11.8 (±1.86) mg N_2O-N kg^{-1} in m+ in contrast to 0.01 (±0.02) mg N_2O-N kg^{-1} in c. Thus, addition of MI to incorporated clover reduced N_2O emission by 27 % which was yet insignificant (p = 0.42) due to one outlier in m.

3.2. Field study

3.2.1. Greenhouse gas fluxes, soil N_{min} and DOC dynamics

Precipitation had a strong influence on N_2O fluxes from cover crop incorporation until maize harvest (Fig. 2). Overall, soil moisture tended to be higher in R than P from cover crop incorporation to the middle of July, except for short periods after rainfall events when moisture levels were the same in both incorporation treatments. Incorporation method had in contrast no influence on soil temperature at 10 cm depth. During the first days after cover crop incorporation, DOC concentrations and CO_2 fluxes increased more in mulched rotary tilled treatments (Rm and Rm+) than they did in all other treatments. DOC and CO_2 were first higher in Rm than in Rm+, with initially no difference between these treatments in N_{min}. Yet, during the first N_2O peak, triggered by a rainfall event on day four after incorporation, the highest N_2O, CO_2 and DOC fluxes were detected in Rm+. At the same time, N_{min} concentration significantly dropped in rotary tilled (R) as compared with ploughed (P) treatments. These parameters also tended to be lower in Rm+ than in Rm.

There were five N_2O peaks between cover crop incorporation and

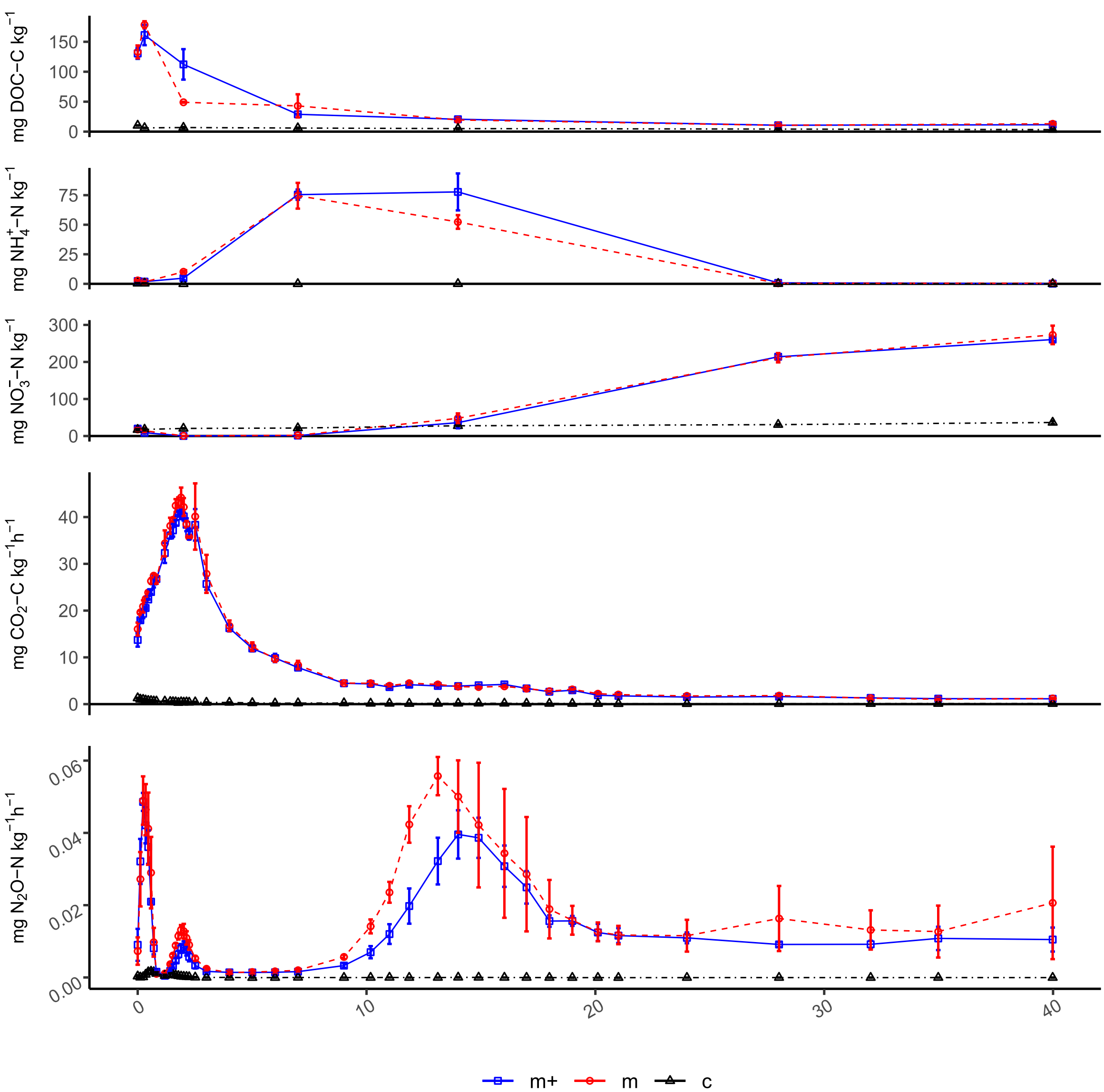

Fig. 1. From top to bottom: Dissolved organic carbon (DOC), ammonium (NH_4^+) and nitrate (NO_3^-) concentrations (mg kg^{-1}) in incubated soils, and CO_2 and N_2O fluxes (mg kg^{-1} h^{-1}) from these soils over the course of a 40-day incubation study. Symbols represent means and error bars standard errors (n = 3). Treatments include clover biomass mixed with soil (m), clover biomass treated with microbial inoculant (m+) and a control with soil only (c).

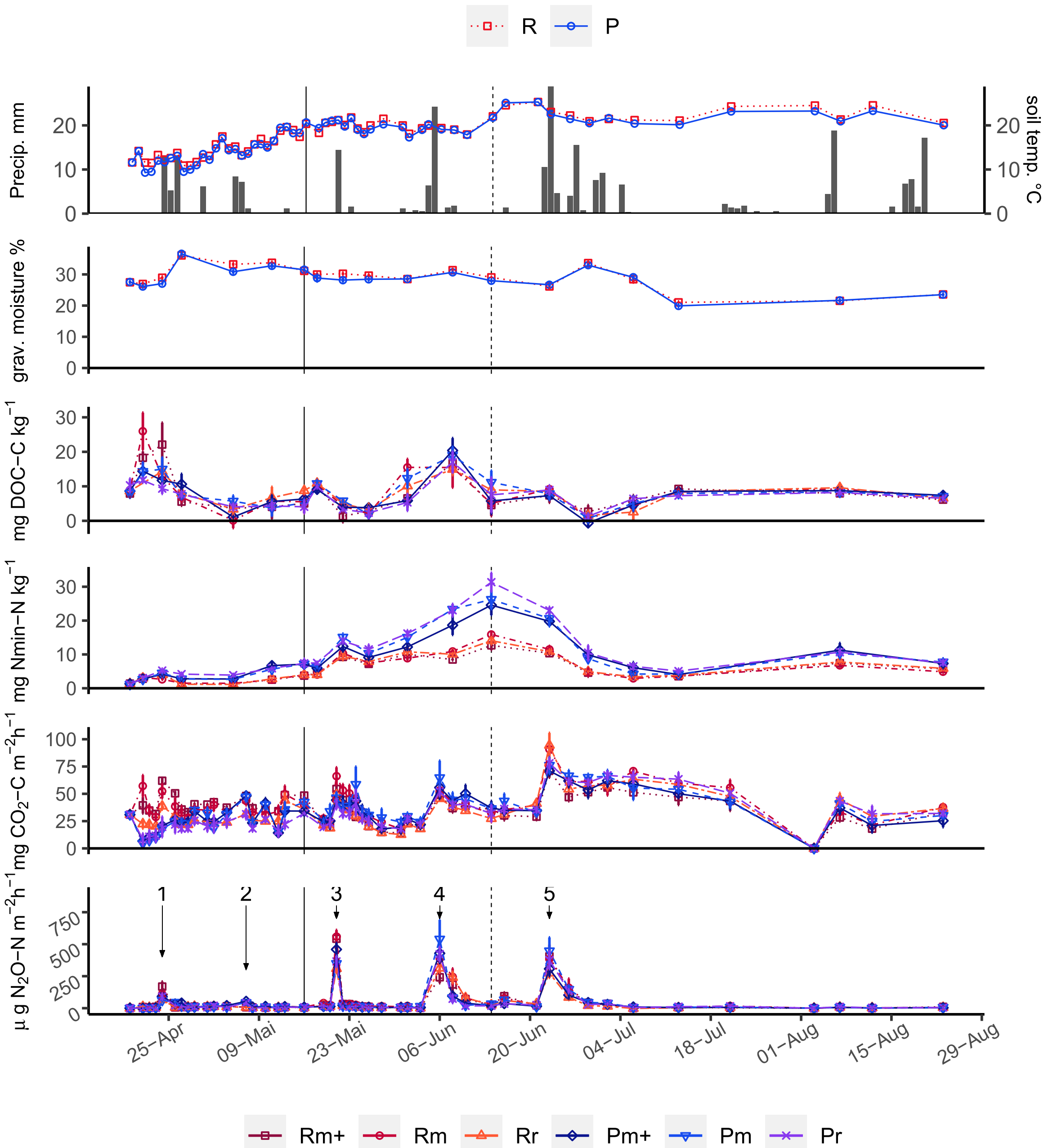

Fig. 2. Time series of observed parameters. From top to bottom: precipitation (mm) and soil temperature (°C, n = 2), mean of gravimetric soil moisture (%, n = 12), mean of available soil nitrogen (N_{min}, nitrate and ammonium, mg kg^{-1}, n = 4), dissolved organic carbon concentrations (DOC, mg kg^{-1}, n = 4), and mean of N_2O and CO_2 fluxes (μg and mg kg^{-1} h-[1], respectively; n = 8) from cover crop incorporation to maize harvest. Symbols represent means and error bar the standard error of the mean. The solid vertical line indicates the time of seedbed preparation and maize sowing; the dashed vertical line that of hoeing and fertilization. Numbers label N_2O peaks. Treatments include superficial rotary tillage (R) versus ploughing (P) and cover crop treatments with aboveground biomass mulched (m), mulched and treated with microbial inoculant (m+) and removed (r).

maize harvest (for peak numbers see Fig. 2). They were all triggered by rainfall. There was a trend to higher N_2O fluxes in R than P after tillage operations (+29 % ($p = 0.13$) for peak 1 and +17 % ($p = 0.40$) for peak 3) whereas during peak 2 and 4, N_2O fluxes were 84 % ($p = 0.03$) and 32 % ($p = 0.13$) higher in P than R. Microbial inoculant showed a tendency to increase N_2O fluxes in Rm+ during the first peak by 0.03 kg N ha^{-1} ($p = 0.29$) and decreased N_2O fluxes in the first half of peak 4 by 0.12 kg N ha^{-1} ($p = 0.35$) in comparison to mulched plots not treated with MI (Rm). Background N_2O fluxes between peaks ranged from 3 to 9 μg N_2O-N m^{-2} h^{-1}. Slurry application did not induce a relevant peak.

3.2.2. Cumulative N_2O and CO_2 emissions

During cover crop decomposition (20 April 2022–16 Ma 2022; Fig. 3A), the removal of the aboveground cover crop biomass had reduced N_2O emissions by 0.034 kg N ha^{-1} ($p = 0.02$) as compared with mulching with MI (m+) across incorporation treatments. In R alone, this accounted for N_2O emissions lowered by 0.047 kg N ha^{-1} ($p = 0.09$) in Rr as compared with Rm+. Cumulative CO_2 emissions were 0.064 kg C ha^{-1} ($p < 0.01$) lower in P than in R (Fig. 3B). Cover crop removal before incorporation reduced CO_2 by 0.048 t C ha^{-1} ($p = 0.04$) in contrast to mulched treatments (m, m+).

Considering the entire season until maize harvest, we found no significant treatment differences in cumulative N_2O emissions (Fig. 4A).

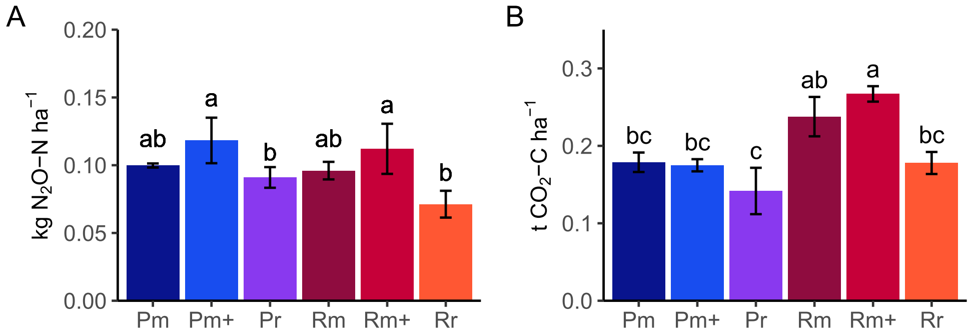

Fig. 3. *: Cumulative N_2O (A, kg N_2O-N ha^{-1}) and CO_2 (B, t CO_2-C ha^{-1})* emissions from cover crop incorporation until maize sowing measured in bare soil (from 20 April 2022–16 Ma 2022). Bars represent means and error bars standard errors (n = 4). Treatments include superficial rotary tillage (R) versus ploughing (P) and cover crop treatments with aboveground biomass mulched (m), mulched and treated with microbial inoculant (m+), and removed (r). Letters indicate significant differences between treatments ($p < 0.05$, Tukey).

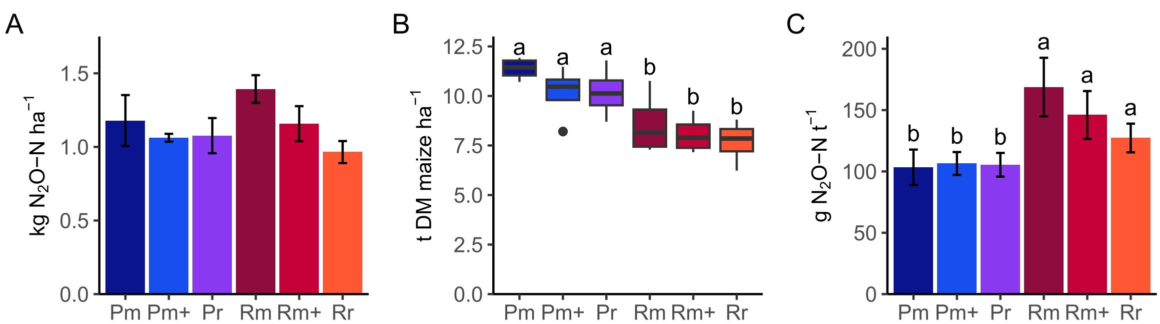

Fig. 4. Cumulative N_2O emissions over the full measurement period (A; from 3 March 2022–23 August 2022, kg N_2O-N ha^{-1}), total aboveground maize biomass (t DM ha^{-1}) and yield-scaled cumulative N_2O emissions (g N_2O-N t^{-1}). Bars represent means and error bars standard errors (n = 4). Treatments include superficial rotary tillage (R) versus ploughing (P), and cover crop treatments with aboveground biomass mulched (m), mulched and treated with microbial inoculant (m+), and removed (r). DM = dry matter. Letters indicate significant differences between treatments ($p < 0.05$, Tukey).

Yet, there was a strong trend towards lower N_2O emissions following the removal (r) of the cover crop aboveground biomass compared to m (-0.27 kg N ha^{-1}, p = 0.06), particularly in rotary tilled plots. Furthermore, there was a weak trend across both incorporation treatments towards lower total N_2O emissions (-0.18 kg N ha^{-1}, p = 0.28) through the application of MI (m+) as compared with mulched plots without MI (m).

3.2.3. Maize yield and yield scaled N_2O emissions

Incorporation method significantly affected maize yield. The aboveground biomass yield was by 2.46 t DM ha^{-1} ($p < 0.01$) higher in P than in R (Fig. 4B). We found neither a positive nor a negative influence of MI or cover crop removal on maize yield. Cumulative yield-scaled N_2O emissions were significantly lower in P than in R, by 42.3 g N t^{-1} of maize yield ($p < 0.01$, Fig. 4C) due to the yield difference.

3.2.4. SOC stocks

Soil organic carbon (SOC) stocks before the trial started in 2021 were on average 66.7 (±3.6) Mg C ha^{-1} in 0–20 cm depth, of which 24.4 (±2.0) Mg C ha^{-1} were in 0–7 cm and 42.3 (±2.7) Mg C ha^{-1} in 7–20 cm depth. After one year, P showed a trend of on average 2.16 Mg C ha^{-1} higher SOC stocks (p = 0.16) compared to R in 0–20 cm depth. In the lower soil layer of 7–20 cm depth, SOC stock changes were significantly larger in P than in R, on average by 3.2 Mg ha^{-1} (p = 0.01, Fig. 5 C).

4. Discussion

4.1. Seasonal effects on N_2O field emissions

Cumulative N_2O emissions over the full crop cycle (173 days) were low and ranged from 0.96 to 1.40 kg N ha^{-1}. The low emissions are linked to dry conditions during cover crop growth, resulting in a low aboveground biomass quantity (2.5 t DM ha^{-1}) that was incorporated, and again little rainfall during maize growth as well as optimal timing of field operations. Measured N_2O emissions were lower than reported N_2O emissions of 2.0–19.0 kg N ha^{-1} between incorporation and maize harvest by Winkhart et al. (2022), where a catch crop mixture and winter rye were incorporated in combination with biogas digestate under more favorable conditions. They were also lower than the 3.6 kg N-N_2O ha^{-1} reported for silage maize grown under organic farming conditions similar to our study (Efosa et al., 2023; Skinner et al., 2019).

In our study, rainfall events exceeding 10 mm combined with a soil N_{min} concentration above 2.5 mg N kg^{-1} and gravimetric soil moisture contents above 25 % triggered N_2O peak emissions.

4.2. Microbial inoculant has a little effect on N_2O and yield

The reduction of total cumulative N_2O emissions by 15 % through the application of microbial inoculant (MI) was statistically not significant (p = 0.28) and lower than the reduction due to the removal of the cover crop in the field assessment. In the 40-day incubation study (T = 20°C, WFPS = 65 %), MI reduced N_2O emissions by 27 %, which was however also statistically not significant. There are few studies available assessing the effect of adding MI during cover crop mulching and incorporation. Liu et al. (2019b) found a similar reduction of N_2O emissions (9–12 %) during the incorporation of rice straw and microbial inoculates with mineral fertilizer before wheat. This difference was also not significant. The composition of microorganisms in their study differed from the MI applied in our study and included *Bacillus subtilis, Pichia pastoris, Rhizopus oryzae* and *Pedioccus pentosaceus* as well as *Bacillus subtilis, Paenibacillus polymyxa, Bacillus brevis* and *Bascillus licheniformis*. And as stated in the introduction, other studies in Asia found no effect or a reduction in N_2O when adding MI to straw in rice or

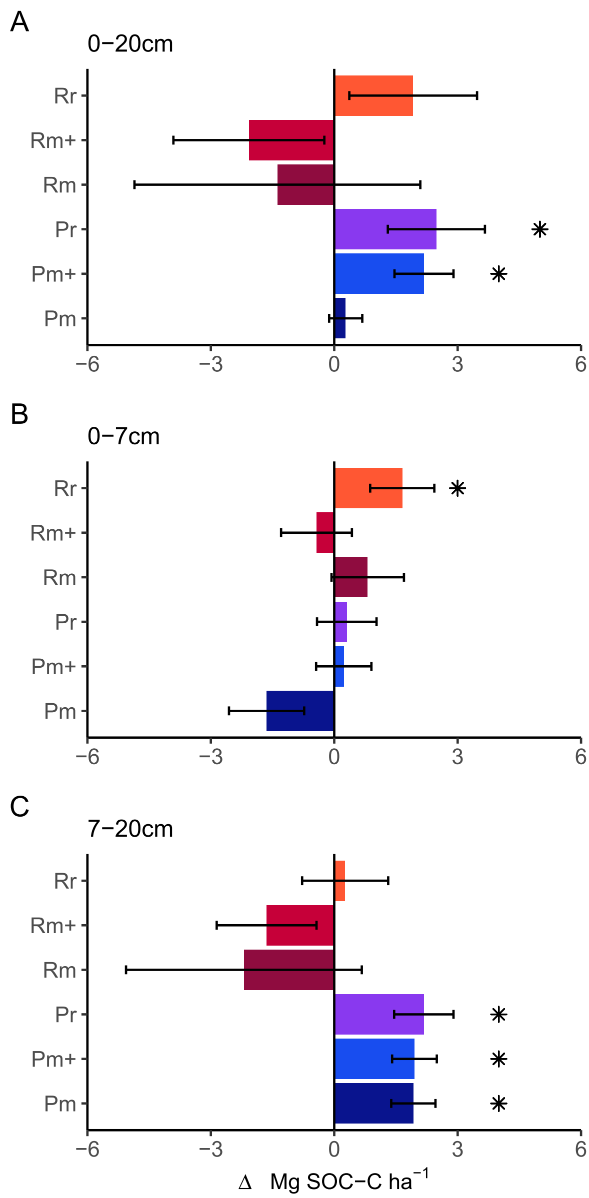

Fig. 5. Changes in soil organic carbon stocks (Mg C ha^{-1}) from 2021 to 2022 in the soil layers 0–20 cm (A), 0–7 cm (B) and 7–20 cm (C). Bars represent means and error bars standard errors (n=4). Treatments include superficial rotary tillage (R) versus ploughing (P) and cover crop treatments with aboveground biomass mulched (m), mulched and treated with microbial inoculant (m+), and removed (r). DM = dry matter. Stars mark significant SOC stock changes between the years ($p < 0.05$, Tukey).

rice-wheat rotations under field conditions (Hao et al., 2022; Liu et al., 2019a, 2015; Ma et al., 2019; Wang et al., 2020). They also reported on potential negative effects by potentially increased CH_4 emissions. Gas samples in our study were analyzed for CH_4. Due to issues with the FID detector, results were however not reliable and thus excluded in this manuscript. In contrast to the temporarily flooded environments in paddy rice systems, soils are aerated in our rotation. In the study of Krauss et al. (2017), which has been done on the same soil in an adjacent field, CH_4 fluxes were mainly negative and the overall uptake in CH_4 minor in terms of the climate mitigation effect than N_2O. We thus also expect a negligible impact of CH_4 emissions in the present study.

In terms of process understanding, MI is claimed by a "regenerative" movement in the German speaking countries to harmonize the decomposition when large amounts of biomass are incorporated into the soil. We therefore sampled with a high frequency after MI application and cover crop incorporation. In the field trial, we found a similar pattern as in the 40-day incubation study, such as higher DOC and N_{min} concentrations and larger N_2O and CO_2 fluxes in MI treatments during the first N_2O-peak. This effect was most pronounced in rotary tilled soils. Microbial inoculant may have increased decomposition of easily degradable organic carbon right after incorporation, consistent with Ney et al. (2018), who reported accelerated N mineralization in the first week after applying a local mix of effective microorganisms in combination with swine effluent.

Furthermore, our data suggest that MI has an influence on the N cycle and thus reduces N_2O formation under certain conditions and during a short time. Lower nitrate and higher ammonium concentration as well as lower N_2O emission in m+ than m during the third peak of the incubation study (after 14 days) suggests that MI may have either inhibited nitrification or increased complete denitrification. We found similar pattern during the fourth peak of the field study. Nevertheless, further studies are needed to support this assumption and to investigate this influence in more detail.

4.3. No clear effects on soil organic carbon stocks

Measurements of SOC stock changes over one year showed a large variation presumably due to soil heterogeneity and the short time interval. Tendentially, SOC stocks increased in ploughed plots between 0 and 20 cm depth. The gain in SOC stock in 7-20 cm likely resulted from burying SOC-rich topsoil that had formed under reduced tillage in the preceding years by ploughing. Average SOC stock changes across all ploughed plots in 0–20 cm of 1.64 Mg C ha^{-1} was similar to the 1.96 Mg C ha^{-1} yr^{-1} reported by Maris et al. (2021), who incorporated different cover crops throughout four years.

Our results accord with common understanding that SOC changes are hardly detectible within such a short time. Long-term measurements indicate an overall positive effect of cover crops on total SOC stocks (Poeplau and Don, 2015). However, it has not yet been well studied how longer-term cover crop management with different incorporation methods affects SOC. The study of Krauss et al. (2022) suggests that in organic farming, shallow reduced tillage of previously ploughed fields mainly induces a redistribution of SOC stocks, where soils under reduced tillage accumulate in the long run SOC in the topsoil. However, total SOC stocks hardly change (Krauss et al., 2022; Poeplau and Don, 2015).

The effect of microbial inoculants on SOC is not well assessed so far. No effects were, e.g., found by Breza-Boruta and Bauza-Kaszewska (2023) on SOC concentrations in 0–30 cm over a period of 3 years. Hu et al. (2018) did also not find a significant difference in SOC stocks between plots fertilized with compost with or without effective microorganisms in 0–30 cm depth over 11 years.

4.4. Cover crop removal reduces N_2O emissions without influencing maize yield

We found similar soil N_{min} dynamics and maize yields irrespective of cover crop treatment. In contrast to our study, Kandel et al. (2020) measured lower NO_3^- concentrations when aboveground parts of a vetch cover crop had been removed before shallow tillage (disk tiller) under even dryer conditions and overall lower NO_3^- concentrations. Their crabgrass yields were 20 % lower when cover crops were removed suggesting that mulched cover crops had contributed to plant nutrition.

In our study, the removal of aboveground parts of the cover crop however reduced N_2O emissions between incorporation and maize sowing by 20 % (in R alone by 30 %) and CO_2 emissions by 20 %. Our study thus confirms that incorporating the entire cover crop biomass can increase field N_2O and CO_2 emissions, as summarized in the meta-analysis by Muhammad et al. (2019). In our case, the cover crop consisted mainly of grass species (75 %), as legumes did not emerge well. The C:N ratio of the aboveground biomass was 26 and the response ratio of N_2O emissions between removed (r) and mulched (m, m+) treatments was 0.87, much higher than the modelled ratio of −0.5 found in the response curve between N_2O emissions and C:N ratio elaborated by Muhammad et al. (2019).

According to our results, removing cover crop biomass seems to be advantageous in the short term. Yet, the removed biomass will be used either as forage, composting material or transfer mulch, which externalizes or postpones greenhouse gas emissions. To evaluate the total impact of different cover crop treatments, the entire chain needs to be considered.

4.5. Ploughing, as compared to rotary tillage, increases maize yield but not N_2O emission

After both tillage events (incorporation of cover crop and sowing of maize), N_2O and CO_2 fluxes in both mulched treatments were higher in R than in P. The rotary tiller shredded cover crop biomass and mixed it with soil more finely than it was done by ploughing. This led to a greater release of easily decomposable biomass in the upper soil layer, measured as higher DOC concentrations in Rm and Rm+ as compared to the other treatments. The increase of DOC after tillage stimulates denitrification (Qin et al., 2017). This may explain the observed decrease in N_{min} concentrations and higher N_2O and CO_2 fluxes in R treatments. The more uniformly mixed cover crop–soil mixture created by rotary tilling most likely also increased cover crop decomposition (increased CO_2 fluxes) compared to the deeper and more aggregated incorporation of biomass in P shortly after incorporation of cover crops. Higher decomposition rates through uniformly mixed biomass was also observed in the incubation study by Loecke and Robertson (2009). In addition, they found up to eight times higher N_2O emissions over 40 days when the biomass was aggregated than uniformly distributed. In contrast to this finding, cumulative N_2O emissions were overall similar between rotary tilled and ploughed plots in our field study. Maize yields were 23 % significantly higher when the soil was ploughed at the same time, most likely due to soil structural issues and different N dynamics in the soil. After rotary tilling, we observed a smearing layer despite working under ideal soil moisture conditions. We thus found higher emissions in R compared to P when N_2O emissions were yield-scaled. Krauss et al. (2017) also found no differences in total area scaled N_2O emissions between reduced and conventional tillage methods after incorporation of two-year grass-clover ley in autumn. Although immediate peak emissions were higher with reduced tillage, increased background emissions in ploughed plots during the rest of the cropping season levelled total N_2O emissions. In contrast, Winkhart et al. (2022) found higher emissions after ploughing of cover crops in two of three years compared to incorporation by reduced tillage. Their more systemic design however linked tillage with cover crop type (legumes and no legumes) and the trial was fertilized just before sowing, which additionally affected decomposition and N_2O emission.

Overall, our data show that different ways of incorporating cover crops could influence climate mitigation by changing N_2O emissions and influencing SOC stocks. Thereby, removal of aboveground cover crop biomass had the greatest impact, followed by incorporation methods and then by MI (Hypothesis II), whereby N_2O reduction through MI in the field was little (Hypothesis I).

5. Conclusions

In this first study that investigated the application of MI to cover crops incorporated by shallow (rotary) tillage or ploughing, we found no significant climate change mitigation effect of MI, but lowered cash crop yields under shallow tillage. Microbial inoculant (MI) appears to have a too little effect on N_2O emissions from agricultural fields to be reliably quantified, even in an elaborate field experiment. Nevertheless, our study revealed consistent changes in N dynamics with MI, which suggest that MI may influence transformation processes after cover crop incorporation. It would be worthwhile to further investigate the influence of MI and clarify which mechanisms exactly caused the observed variations. Perhaps new insights can be gained at the microbial level that help to understand N_2O emission pathways in a broader context. So far, it remains open whether MI might potentially reduce the contribution of agriculture to climate change.

CRediT authorship contribution statement

Sebastian Rieder: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Franz Conen:** Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Maike Krauss:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maike Krauss reports financial support was provided by Bio Suisse, Sur-la-Croix Foundation, Canton of Basel Country, Canton of Basel City, Canton Aargau, Canton of Solothurn, Swiss Confederation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.109332.

Data availability

Data will be made available on request.

References

Abalos, D., Recous, S., Butterbach-Bahl, K., De Notaris, C., Rittl, T.F., Topp, C.F.E., Petersen, S.O., Hansen, S., Bleken, M.A., Rees, R.M., Olesen, J.E., 2022a. A review and meta-analysis of mitigation measures for nitrous oxide emissions from crop residues. Sci. Total Environ. 828, 154388. https://doi.org/10.1016/j.scitotenv.2022.154388.

Abalos, D., Rittl, T.F., Recous, S., Thiébeau, P., Topp, C.F.E., van Groenigen, K.J., Butterbach-Bahl, K., Thorman, R.E., Smith, K.E., Ahuja, I., Olesen, J.E., Bleken, M.A., Rees, R.M., Hansen, S., 2022b. Predicting field N2O emissions from crop residues based on their biochemical composition: a meta-analytical approach. Sci. Total Environ. 812, 152532. https://doi.org/10.1016/j.scitotenv.2021.152532.

Abdalla, M., Osborne, B., Lanigan, G., Forristal, D., Williams, M., Smith, P., Jones, M.B., 2013. Conservation tillage systems: a review of its consequences for greenhouse gas emissions. Soil Use Manag. 29, 199–209. https://doi.org/10.1111/sum.12030.

Basche, A.D., Miguez, F.E., Kaspar, T.C., Castellano, M.J., 2014. Do cover crops increase or decrease nitrous oxide emissions? A meta-analysis. J. Soil Water Conserv. 69, 471–482. https://doi.org/10.2489/jswc.69.6.471.

Böldt, M.J., Smit, H.P.J., Loges, R., Taube, F., Kluß, C., Reinsch, T., 2024. Evaluating nitrous oxide emissions in low input systems using different cover crop strategies over the winter period. Agric., Ecosyst. Environ. 364, 108895. https://doi.org/10.1016/j.agee.2024.108895.

Breza-Boruta, B., Bauza-Kaszewska, J., 2023. Effect of Microbial Preparation and Biomass Incorporation on Soil Biological and Chemical Properties. Agriculture 13, 969. https://doi.org/10.3390/agriculture13050969.

Efosa, N., Krause, H.-M., Hüppi, R., Krauss, M., Vaucher, N., Zourek, F., Mayer, J., Six, J., Bünemann, E.K., 2023. Emissions of nitrous oxide and methane after field application of liquid organic fertilizers and biochar. Agric., Ecosyst. Environ. 356, 108642. https://doi.org/10.1016/j.agee.2023.108642.

Eurostat, 2016. Share of tillage practices in the EU27 2016 [WWW Document]. URL https://ec.europa.eu/eurostat/statistics-explained/index.php?title=File:Figure1_Share_of_tillage_practices_in_the_EU27_2016.png (accessed 11.5.23).

Flessa, H., Dörsch, P., Beese, F., 1995. Seasonal variation of N2O and CH4 fluxes in differently managed arable soils in southern Germany. J. Geophys. Res. Atmos. 100, 23115–23124. https://doi.org/10.1029/95JD02270.

Fontana, M., Berner, A., Mäder, P., Lamy, F., Boivin, P., 2015. Soil Organic Carbon and Soil Bio-Physicochemical Properties as Co-Influenced by Tillage Treatment. Soil Sci. Soc. Am. J. 79, 1435–1445. https://doi.org/10.2136/sssaj2014.07.0288.

Guenet, B., Gabrielle, B., Chenu, C., Arrouays, D., Balesdent, J., Bernoux, M., Bruni, E., Caliman, J., Cardinael, R., Chen, S., Ciais, P., Desbois, D., Fouche, J., Frank, S., Henault, C., Lugato, E., Naipal, V., Nesme, T., Obersteiner, M., Pellerin, S., Powlson, D.S., Rasse, D.P., Rees, F., Soussana, J., Su, Y., Tian, H., Valin, H., Zhou, F., 2021. Can N_2O emissions offset the benefits from soil organic carbon storage? Glob. Change Biol. 27, 237–256. https://doi.org/10.1111/gcb.15342.

Haddaway, N.R., Hedlund, K., Jackson, L.E., Kätterer, T., Lugato, E., Thomsen, I.K., Jørgensen, H.B., Isberg, P.-E., 2017. How does tillage intensity affect soil organic carbon? A systematic review. Environ. Evid. 6, 30. https://doi.org/10.1186/s13750-017-0108-9.

Hansen, S., Berland Frøseth, R., Stenberg, M., Stalenga, J., Olesen, J.E., Krauss, M., Radzikowski, P., Doltra, J., Nadeem, S., Torp, T., Pappa, V., Watson, C.A., 2019. Reviews and syntheses: review of causes and sources of N₂O emissions and NO₃ leaching from organic arable crop rotations. Biogeosciences 16, 2795–2819. https://doi.org/10.5194/bg-16-2795-2019.

Hao, M., Guo, L.-J., Du, X., Wang, H.-L., Sheng, F., Li, C.-F., 2022. Integrated effects of microbial decomposing inoculant on greenhouse gas emissions, grain yield and economic profit from paddy fields under different water regimes. Sci. Total Environ. 805, 150295. https://doi.org/10.1016/j.scitotenv.2021.150295.

Hu, C., Xia, X., Chen, Y., Han, X., Hu, C., Xia, X., Chen, Y., Han, X., 2018. Soil carbon and nitrogen sequestration and crop growth as influenced by long-term application of effective microorganism compost. Chil. J. Agric. Res. 78, 13–22. https://doi.org/10.4067/S0718-58392018000100013.

Hu, T., Sørensen, P., Olesen, J.E., 2018. Soil carbon varies between different organic and conventional management schemes in arable agriculture. Eur. J. Agron. 94, 79–88. https://doi.org/10.1016/j.eja.2018.01.010.

Hüppi, R., Felber, R., Krauss, M., Six, J., Leifeld, J., Fuß, R., 2018. Restricting the nonlinearity parameter in soil greenhouse gas flux calculation for more reliable flux estimates. PLOS ONE 13, e0200876. https://doi.org/10.1371/journal.pone.0200876.

Kandel, T.P., Gowda, P.H., Northup, B.K., Rocateli, A.C., 2020. Incorporation and harvest management of hairy vetch-based green manure influence nitrous oxide emissions. Renew. Agric. Food Syst. 35, 561–570. https://doi.org/10.1017/S174217051900019X.

Krauss, M., Ruser, R., Müller, T., Hansen, S., Mäder, P., Gattinger, A., 2017. Impact of reduced tillage on greenhouse gas emissions and soil carbon stocks in an organic grass-clover ley - winter wheat cropping sequence. Agric., Ecosyst. Environ. 239, 324–333. https://doi.org/10.1016/j.agee.2017.01.029.

Krauss, M., Wiesmeier, M., Don, A., Cuperus, F., Gattinger, A., Gruber, S., Haagsma, W. K., Peigné, J., Palazzoli, M.C., Schulz, F., van der Heijden, M.G.A., Vincent-Caboud, L., Wittwer, R.A., Zikeli, S., Steffens, M., 2022. Reduced tillage in organic farming affects soil organic carbon stocks in temperate Europe. Soil Tillage Res. 216, 105262. https://doi.org/10.1016/j.still.2021.105262.

Leifeld, J., Ammann, C., Neftel, A., Fuhrer, J., 2011. A comparison of repeated soil inventory and carbon flux budget to detect soil carbon stock changes after conversion from cropland to grasslands. Glob. Change Biol. 17, 3366–3375. https://doi.org/10.1111/j.1365-2486.2011.02471.x.

Liu, G., Yu, H., Ma, J., Xu, H., Wu, Q., Yang, J., Zhuang, Y., 2015. Effects of straw incorporation along with microbial inoculant on methane and nitrous oxide emissions from rice fields. Sci. Total Environ. 518–519 209–216. https://doi.org/10.1016/j.scitotenv.2015.02.028.

Liu, G., Ma, J., Yang, Y., Yu, H., Zhang, G., Xu, H., 2019a. Effects of straw incorporation methods on nitrous oxide and methane emissions from a wheat-rice rotation system. Pedosphere 29, 204–215. https://doi.org/10.1016/S1002-0160(17)60410-7.

Liu, G., Ma, J., Yang, Y., Yu, H., Zhang, G., Xu, H., 2019b. Effects of straw incorporation methods on nitrous oxide and methane emissions from a wheat-rice rotation system. Pedosphere 29, 204–215. https://doi.org/10.1016/S1002-0160(17)60410-7.

Loecke, T.D., Robertson, G.P., 2009. Soil resource heterogeneity in terms of litter aggregation promotes nitrous oxide fluxes and slows decomposition. Soil Biol. Biochem. 41, 228–235. https://doi.org/10.1016/j.soilbio.2008.10.017.

Ma, Y., Liu, D.L., Schwenke, G., Yang, B., 2019. The global warming potential of straw-return can be reduced by application of straw-decomposing microbial inoculants and biochar in rice-wheat production systems. Environ. Pollut. 252, 835–845. https://doi.org/10.1016/j.envpol.2019.06.006.

Maris, S.C., Fiorini, A., Boselli, R., Santelli, S., Tabaglio, V., 2021. Cover crops, compost, and conversion to grassland to increase soil C and N stock in intensive agrosystems. Nutr. Cycl. Agroecosyst. 119, 83–101. https://doi.org/10.1007/s10705-020-10110-9.

Muhammad, I., Sainju, U.M., Zhao, F., Khan, A., Ghimire, R., Fu, X., Wang, J., 2019. Regulation of soil CO2 and N2O emissions by cover crops: a meta-analysis. Soil Tillage Res. 192, 103–112. https://doi.org/10.1016/j.still.2019.04.020.

Ney, L., Franklin, D., Mahmud, K., Cabrera, M., Hancock, D., Habteselassie, M., Newcomer, Q., 2018. Examining trophic-level nematode community structure and nitrogen mineralization to assess local effective microorganisms' role in nitrogen availability of swine effluent to forage crops. Appl. Soil Ecol. 130, 209–218. https://doi.org/10.1016/j.apsoil.2018.06.015.

Pinheiro, J., Bates, D., R Core Team, 2020. nlme: Linear and Nonlinear Mixed Effects Models. http://CRAN.R-project.org/package=nlme.

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. Agric. Ecosyst. Environ. 200, 33–41. https://doi.org/10.1016/j.agee.2014.10.024.

Qin, S., Hu, C., Clough, T.J., Luo, J., Oenema, O., Zhou, S., 2017. Irrigation of DOC-rich liquid promotes potential denitrification rate and decreases N2O/(N2O+N2) product ratio in a 0–2 m soil profile. Soil Biol. Biochem. 106, 1–8. https://doi.org/10.1016/j.soilbio.2016.12.001.

Regenerativ Schweiz, 2023. Regenerative Landwirtschaft [WWW Document]. Regenerativ Schweiz. URL https://en.regenerativ.ch/regenerative-landwirtschaft (accessed 10.28.23).

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rogelj, J., Luderer, G., Pietzcker, R.C., Kriegler, E., Schaeffer, M., Krey, V., Riahi, K., 2015. Energy system transformations for limiting end-of-century warming to below 1.5 °C. Nat. Clim. Change 5, 519–527. https://doi.org/10.1038/nclimate2572.

Schwab, P., Gubler, A., 2016. Methoden zur Bestimmung physikalischer Begleitparameter an Bodenproben. Agroscope Sci. 34.

Seneviratne, S.I., Rogelj, J., Séférian, R., Wartenburger, R., Allen, M.R., Cain, M., Millar, R.J., Ebi, K.L., Ellis, N., Hoegh-Guldberg, O., Payne, A.J., Schleussner, C.-F., Tschakert, P., Warren, R.F., 2018. The many possible climates from the Paris Agreement's aim of 1.5 °C warming. Nature 558, 41–49. https://doi.org/10.1038/s41586-018-0181-4.

Skinner, C., Gattinger, A., Krauss, M., Krause, H.-M., Mayer, J., van der Heijden, M.G.A., Mäder, P., 2019. The impact of long-term organic farming on soil-derived greenhouse gas emissions. Sci. Rep. 9, 1702. https://doi.org/10.1038/s41598-018-38207-w.

von Haden, A.C., Yang, W.H., DeLucia, E.H., 2020. Soils' dirty little secret: Depth-based comparisons can be inadequate for quantifying changes in soil organic carbon and other mineral soil properties. Glob. Change Biol. 26, 3759–3770. https://doi.org/10.1111/gcb.15124.

Wang, L., Qin, T., Liu, T., Guo, L., Li, C., Zhai, Z., 2020. Inclusion of microbial inoculants with straw mulch enhances grain yields from rice fields in central China. Food Energy Secur. 9, e230. https://doi.org/10.1002/fes3.230.

Winkhart, F., Mösl, T., Schmid, H., Hülsbergen, K.-J., 2022. Effects of organic maize cropping systems on nitrogen balances and nitrous oxide emissions. Agriculture 12, 907. https://doi.org/10.3390/agriculture12070907.