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Soil aggregate stability governs field greenhouse gas fluxes in agricultural soils

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ABSTRACT

Agriculture is responsible for 30–50% of the yearly CO_2, CH_4, and N_2O emissions. Soils have an important role in the production and consumption of these greenhouse gases (GHGs), with soil aggregates and the inhabiting microbes proposed to function as biogeochemical reactors, processing these gases. Here we studied, for the first time, the relationship between GHG fluxes and aggregate stability as determined via laser diffraction analysis (LDA) of agricultural soils, as well as the effect of sustainable agricultural management strategies thereon. Using the static chamber method, all soils were found to be sinks for CH_4 and sources for CO_2 and N_2O. The application of organic amendments did not have a conclusive effect on soil GHG fluxes, but tilled soils emitted more CO_2. LDA was a useful and improved method for assessing soil aggregate stability, as it allows for the determination of multiple classes of aggregates and their structural composition, thereby overcoming limitations of traditional wet sieving. Organic matter content was the main steering factor of aggregate stability. The presence of persistent stable aggregates and the disintegration coefficient of stable aggregates were improved in organic-amended and no-tilled soils. Predictive modelling showed that, especially in these soils, aggregate stability was a governing factor of GHG fluxes. Higher soil CH_4 uptake rates were associated with higher aggregate stability, while CO_2 and N_2O emissions increased with higher aggregate stability. Altogether, it was shown that sustainable agricultural management strategies can be used to steer the soil's aggregate stability and, both consequently and outright, the soil GHG fluxes, thereby creating a potential to contribute to the mitigation of agricultural GHG emissions.

1. Introduction

Earth's most important greenhouse gases (GHGs) are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Friedlingstein et al., 2022; Saunois et al., 2020; Tian et al., 2020), and the current atmospheric concentrations of 410 parts per million (ppm_v) for CO_2, 1.896 ppm_v for CH_4, and 332 parts per billion (ppb_v) for N_2O are the highest in at least 800,000 years (IPCC, 2021). Agricultural activities are estimated to contribute approximately 30–50% to the yearly emissions of these GHGs and are as such one of the main contributors to global warming (IPCC, 2021; Ren et al., 2017; Saunois et al., 2020; Tian et al., 2020). Strong, rapid and sustained reductions in these emissions are thus needed to limit their global warming effects (IPCC, 2021, 2022).

Soils have an important role in the regulation of GHG emissions and the underlying biological processes, as they are capable of both emitting and absorbing these gases (Tian et al., 2016). Climate-smart sustainable agriculture aims to minimize GHG emissions by enhancing carbon sequestration while maintaining or even enhancing soil fertility and productivity (Carlson et al., 2017; Paustian et al., 2016). Soil CO_2 emission is a product of the decomposition of organic carbon and soil

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respiration, which includes anaerobic and aerobic microbial respiration, and can be substantial. It is often increased as a result of land-use change and agricultural use, as fertilization enhances soil respiration (Friedlingstein et al., 2022; Lokupitiya and Paustian, 2006; Oertel et al., 2016). Previously, it has been shown that the carbon sequestration potential of agricultural soils can be improved whilst decreasing CO_2 emissions by using climate-smart agricultural management strategies such as non-inversion tillage and organic amendments (Cooper et al., 2021; Freibauer et al., 2004; Lokupitiya and Paustian, 2006; Ruis et al., 2022). In soils, CH_4 is produced under anaerobic conditions via methanogenesis by methanogenic Archaea as the end product of organic matter decomposition or the reduction of CO_2, acetate, and other C_1-compounds. The produced CH_4 can be emitted, or consumed as carbon or energy source by methane oxidizing bacteria (MOB), also known as methanotrophs, in oxic upland soils generally under aerobic conditions (Conrad, 1996; Guerrero-Cruz et al., 2021). Soils are as yet the only known biological sink for CH_4 (Saunois et al., 2020), but as a result of conventional agricultural practices like ploughing and the use of nitrogen-rich mineral fertilizers, the CH_4 consumption of agricultural soils can be reduced 3 to 9 times compared to undisturbed upland soils (Ho et al., 2015; Levine et al., 2011). However, the use of organic amendments like compost or crop residue incorporation can significantly enhance the methane uptake potential of agricultural soils (Brenzinger et al., 2021; Ho et al., 2015, 2017, 2019). It has been hypothesized that this stimulation is because of the promotion of internal CH_4 production by anaerobic conditions in micro-spots in the soil due to enhanced respiration after organic carbon addition. Hereby, MOB are activated and fueled with energy to co-oxidize atmospheric CH_4 together with the internally produced CH_4 (Bodelier et al., 2019). Soil N_2O emissions are mainly driven by the widespread use of nitrogen-rich mineral fertilizer (Bodelier and Steenbergh, 2014; Butterbach-Bahl et al., 2013; Mosier et al., 1998; Reay et al., 2012), making agriculture the dominant anthropogenic N_2O source (Reay et al., 2012; Tian et al., 2020). N_2O production is controlled by two key biochemical processes, nitrification and denitrification. These processes can occur simultaneously in soils, albeit in different micro-habitats, and are among others dependent on the oxygen availability (Butterbach-Bahl et al., 2013; Skinner et al., 2014; Tian et al., 2020). The use of organic amendments can lead to increased N_2O emissions, as the mineral nitrogen input can be nitrified and subsequently denitrified (Charles et al., 2017). However, as yet, no consistent effect of sustainable agricultural management strategies has been found on soil N_2O emissions (Bayer et al., 2016; Gregorich et al., 2005; Meng et al., 2005; Skinner et al., 2019), indicating the variable nature of agricultural soil N_2O fluxes.

Soils are considered as dynamic, heterogeneous, and biologically active matrices, with their functioning linked to their textural composition or three-dimensional architecture, and structural arrangement of solid particles and pores (Sullivan et al., 2022). The interconnected structure of soil pores, where individual pores can vary in size and shape from micropores to macropores, allows for the diffusion of atmospheric and soil gases through the soil (Ball, 2013; Hartmann and Six, 2022). When the pore space is filled with water, for instance during wet environmental conditions, the total pore volume will be decreased, hampering gas diffusion through the soil (Du et al., 2023; Kuncoro et al., 2014; Sullivan et al., 2022). Furthermore, organo-mineral associations and mineral particles concurrently aggregate and disaggregate by physicochemical and chemical interactions to form complex compound soil structures known as soil aggregates (Totsche et al., 2018; Yudina and Kuzyakov, 2023). Aggregates exhibit physical, chemical and biological properties that differ from the bulk soil and neighboring particles within the soil matrix (Hernandez-Soriano et al., 2018; Or et al., 2021; Regelink et al., 2015; Wang et al., 2019a; Yudina and Kuzyakov, 2019, 2023). The soil aggregate structure consists of macroaggregates (>250 μm) and microaggregates (<250 μm), which can be subdivided into large microaggregates (50–250 μm), small microaggregates (2–50 μm), and (composite) building units (<2 μm) (Totsche et al., 2018). Small microaggregates assemble into progressively larger macroaggregates, and microaggregates can even form within macroaggregates (Six et al., 2000; Wilpiszeski et al., 2019).

Aggregates play an important role in various soil functions, like water and carbon storage, maintenance of genetic diversity, and biogeochemical cycling (Banwart et al., 2019; Hartmann and Six, 2022; Rabbi et al., 2016; Totsche et al., 2018). Wang et al. (2019a) proposed a conceptual model of soil aggregates as biogeochemical reactors involved in GHG dynamics based on the dual nature of soil aggregates (the unity of solids and pores), allowing for gaseous diffusion in and out of soil aggregates (Yudina and Kuzyakov, 2023). Organic matter can become occluded and stabilized within aggregates (Angst et al., 2017; Six et al., 2002), and oxygen, water, and nutrients among others, can diffuse into aggregates, creating individual aggregate-specific environmental circumstances. More importantly, as a result of the oxygen consumption for microbial activity within an aggregate, aggregates can experience oxygen limitation creating anoxic (micro-)spots, generally in the aggregates' core, even in well-aerated soils (Ebrahimi and Or, 2016, 2018). This enables a variety of (anaerobic) metabolic processes to occur within aggregates, including CH_4, N_2O and CO_2 production (Sihi et al., 2020; Wang et al., 2019a). The proposition of soil aggregates as biogeochemical reactors is well-debated (Baveye, 2020; Kravchenko et al., 2019; Wang et al., 2019b), and is agreed upon to be essential for understanding processes concerning gaseous diffusion (Yudina and Kuzyakov, 2019).

As soil aggregates thus play a pivotal role in soil functioning, determining their stability is essential (Baveye et al., 2018; Rabot et al., 2018; Totsche et al., 2018). Aggregate stability is traditionally approached via wet sieving (Kemper, 1965), although this method faces limitations like the lack of repeatability and the limited size range of measurable aggregates (Gyawali and Stewart, 2019). The analysis of aggregate stability using laser diffraction analysis (LDA) (Kasmerchak et al., 2019; Mason et al., 2011; Xue et al., 2021) offers a contemporary and quantitative perspective of aggregate stability, due to the continuous assessment of the disintegration of aggregates of all sizes and classes <2 mm.

Organic agricultural management strategies using organic amendments like compost or crop residue incorporation, can improve the soil organic matter content, the aggregation, and aggregate stability of agricultural soils (Bucka et al., 2019; Hoffland et al., 2020; Lehtinen et al., 2014; Saleem et al., 2020; Totsche et al., 2018). It has been shown before that aggregate size and stability as determined via wet sieving significantly affects GHG fluxes in soils (Dowdeswell-Downey et al., 2023; Jiang et al., 2021; Mangalassery et al., 2013; Stegarescu et al., 2020; Xu et al., 2021), but the relationships between GHG fluxes and the stability of aggregates on the basis of LDA remain to be elucidated. In this study, the effects of different climate-smart management strategies (organic amendments vs. no fertilizer, and conventional tillage vs. non-inversion tillage) were explored for (1) *in situ* soil GHG fluxes and (2) laser diffraction analysis determined soil aggregate stability. The most important regulating factors of soil GHG fluxes and aggregate stability were unraveled using correlative and multivariate modelling, offering valuable insights on the GHG mitigation potential of agricultural soils and climate-smart agricultural management strategies.

2. Material and methods

2.1. Field site description and soil sampling

Six long-term field experiments (>5 years) in the Netherlands, Belgium and Switzerland were selected for the field study. All are randomized block-design field sites with a non-fertilized control and an organic-amendment treatment (for 5 locations compost-amended and for 1 location cover crops-incorporated). The sites represent common soil types and agricultural practices (regular tillage and non-inversion tillage (<5 cm), hereafter referred to as no-tillage) for northwestern

Table 1
Field site description and sampling information.

Location	Coordinates	Sampling date	Agricultural practice	Treatments	Application amount	Time since application	Application yearly since	Soil type	Plot cover	Additional comments	References
Vredepeel, Netherlands	51.542934, 5.849262	2-4-2019	No-tillage and tillage	Non-fertilized control and compost-amended	10 ton ha^{-1} yr^{-1}	14 days	2011	Sandy soil (sand 93.3%, silt 4.5%, clay 2.2%)	Grass-clover	none	de Haan et al. (2018); Quist et al. (2016); Schrama et al. (2018)
Valthermond, Netherlands	52.877470, 6.929775	15-4-2019	No-tillage	Non-fertilized control and compost-amended	20 ton ha^{-1} yr^{-1}	12 days	2013	Sandy peat soil (sand 90%, silt 7%, clay 3%)	Bare soil	Reclaimed peat, now sandy soil	de Haan et al. (2020); Harkes et al. (2019)
Lelystad, Netherlands	52.545608, 5.578861	30-4-2019	No-tillage	Non-fertilized control and compost-amended	20 ton ha^{-1} yr^{-1}	41 days	2012	Clay loam soil (sand 66%, silt 12%, clay 23%)	Weeds	Weed control using Roundup Ultimate (3 L ha^{-1}) 30 days prior	Crittenden et al. (2014)
Melle, Belgium	50.979721, 3.816310	14-5-2019	Tillage	Non-fertilized control and compost-amended	50 m^3 ha^{-1} yr^{-1} (≈20 ton ha^{-1} yr^{-1})	7 days	2010	Loamy sand soil (sand 79.8%, silt 11.6%, clay 8.6%)	Fodder beet or forage maize	Measurements in between crop rows. Yearly 200 kg ha^{-1} nitrogen fertilization for all treatments	D'Hose et al. (2012)
Wageningen, Netherlands	51.995069, 5.659699	16-3-2020	Tillage	Non-fertilized control and cover crops-incorporated	approx. 5 ton ha^{-1} yr^{-1}	14 days	2016	Sandy soil (sand 83%, silt 12%, clay 2%)	Bare soil	Cover crop mixture (1:1:1) of *Raphanus sativus* (radish), *Avena strigosa* (oat), and *Vicia sativa* (vetch)	Drost (2022); Elhakeem et al. (2023)
Zürich, Switzerland	47.428737, 8.516487	12-10-2021	Tillage	Non-fertilized control and compost-amended	2.5 ton dry org. matter ha^{-1} yr^{-1} (approx. 8 ton ha^{-1} yr^{-1})	8 days	1949	Sandy loam soil (sand 57%, silt 27%, clay 14%)	Bare soil	Weed and pest control using herbicides and fungicides during whole growing season (last 55 days prior)	Oberholzer et al. (2014)

Europe (Table 1).

Soil temperature, humidity, and pore water conductivity were determined on every plot as an average of three measurements using a W.E.T. sensor (Royal Eijkelkamp B.V., Giesbeek, The Netherlands). Soil samples (0–15 cm depth) were collected following a consequent pattern covering the complete plot using a gouge auger, and subsequently air-dried for 3 weeks after gently breaking up large clods along natural fissures into natural aggregates. The airdried samples were non-destructively sieved <2 mm whilst applying the least amount of physical pressure possible, and stored at room temperature until further analysis. Organic matter content was measured as loss of weight on ignition, by first drying samples at 105 °C for 24 h and subsequently burning them in an oven at 550 °C for 4 h (Dean, 1974).

2.2. *Greenhouse gas fluxes*

Field GHG fluxes were measured using custom-built static opaque PVC chambers with a battery-powered internal ventilator, a volume of 28 L and a surface area of 0.071 m^2. To this end, on every plot, a chamber was mounted on a PVC ring that was inserted 5–10 cm in the soil and was sealed off airtightly with an internal rubber sealing. Insulation foil was used to cover the chambers to prevent an increase of the temperature inside the chamber during measurements (see figure 5.4 of Drost, 2022). The chambers were closed for approximately 3.5 h. Headspace samples (60 mL) were collected at the start and end of the measurement period, and at regular intervals in between, using a disposable syringe. Roughly 54 mL of sample was used to flush a 6 mL exetainer vial (Labco Limited, Lampeter, United Kingdom), after which the remaining 6 mL sample was injected in the exetainer vial, creating a 1 bar overpressure. The vials were stored at room temperature until further analysis.

A TriPlus RSH autosampler (Thermo Fisher Scientific, Bleiswijk, the Netherlands) was used to introduce 2 mL of sample into a GC1300 gas chromatograph (GC) (Interscience, Breda, the Netherlands) equipped with a Methanizer and a Flame Ionization Detector (FID) to detect CO_2 and CH_4, and an Electron Capture Detector (ECD) to detect N_2O. The GC contained two sets of a pair Rt-Q-Bond capillary columns (L 15 + 30 m, ID 0.53 mm, df 20 μm; Restek, Interscience). Nitrogen was used as a carrier gas and oven temperature was set at 35 °C. Five different concentrations of CH_4 (0.1, 0.2, 0.6, 1.2, 2 ppm_v), CO_2 (100, 200, 600, 1200, 2000 ppm_v), and N_2O (0.05, 0.1, 0.3, 0.6, 1.0 ppm_v) from a gas mixture (2 ppm_v CH_4, 2000 ppm_v CO_2,1 ppm_v N_2O) (Linde Gas Benelux, Velsen-Noord, The Netherlands) were used as a standard. Chromeleon™ Chromatography Data System 7.2.10 software (Thermo Fisher Scientific) was used to process the obtained chromatograms from the GC. The GHG flux rates were determined by linear regression of accumulation or depletion curves for all three gases ($p \leq 0.05$). A substitute flux rate based on the limit of detection of the GC was calculated for curves with a flux rate below said detection limit and for curves with a regression of $p > 0.05$.

2.3. *Laser diffraction analysis of soil aggregate stability*

The size distribution of particles smaller than 2 mm, hereafter called the dry dispersion (DD), was determined using a Mastersizer 3000 Laser Diffraction Analyzer equipped with an Aero S Dry Dispersion Unit and Aero Funnel Sample Feeder (Malvern Panalytical, Malvern, United Kingdom). The refractive data was analyzed using the Mastersizer software (version 3.81). The ‘general purpose’ model for ‘non-spherical particles’ was used, and the calculation of % by volume was based on the Mie theory (de Boer et al., 1987), using a refractive index of 1.52 and an absorption coefficient of 0.1. Six to eight technical replicates were measured and approximately 5–10 g of soil per replicate was used per measurement to reach a minimum obscuration value of 2 % with a dispersive air pressure of 0.1 bar.

Aggregate stability was determined using an adjusted protocol based on the methods described by Kasmerchak et al. (2019) and Mason et al. (2011), foremostly by reducing measurement time to make the method more feasible, and secondly by extending the parameters to all size classes to get a more complete picture of the soil aggregate stability. To this end, a Mastersizer 3000 Laser Diffraction Analyzer equipped with a Hydro LV Dispersion Unit (Malvern Panalytical) was used, and the refractive data was analyzed using the same software and settings as described above. Three technical replicates per sample were measured, and approximately 300 mg (clayey soils) or 1000 mg (sandy soils) per replicate was loaded in the dispersion unit. Measurements were done in demineralized water, and performed every minute during 90 min of continued water suspension and -circulation. A measurement consisted of 20 s of measuring diffraction with a red light source (max. 4 mW He–Ne, 632.8 nm) and subsequently 10 s with a blue light source (max. 10 mW LED, 470 nm), followed by a cool-off period of 30 s. After 90 measurements, 5 min of sonication at 100% was performed to break up and disintegrate all remaining aggregates, and 10 mL of sodium metaphosphate (50 g/L) was added to prevent re-aggregation. The full dispersion (FD) was determined as the average of 5 measurements after sonication.

For both dry dispersion and aggregate stability measurements, refractive data with an obscuration of <2 % or >25 % were discarded, all transformed raw data were exported to Microsoft Excel and downstream analysis was performed using R (version 4.2.1) (R Core Team, 2020). To assess aggregate stability, the increase in particles of the <20 μm size fraction was used. The transformed raw data was curve-fitted using the following equation from Kasmerchak et al. (2019):

$$\text{(Eq. 1)}\ \% < 20\mu m = A_{0,1}\left(1 - e^{-k_1 t}\right) + A_{0,2}\left(1 - e^{-k_2 t}\right)$$

which assumes the presence of two distinct populations of aggregates in the soil sample, weak and stable aggregates. The *changepoint* package in R (Killick and Eckley, 2014) was used to determine the changepoint in both the mean and variance between the two different populations of aggregates, instead of estimating it using least squares. The curve-fit modelling of the transformed raw data to this formula resulted in multiple parameters to extensively describe aggregate stability and disintegration kinetics of the soil (Fig. 1 and Table 2). Furthermore, the presence of persistent stable aggregates, defined as aggregates that are persisting during 90 min of water suspension and -circulation (Kasmerchak et al., 2019), was assessed for the size classes <2 μm, <20 μm, 20–50 μm, 50–250 μm, and >250 μm. Additionally, the presence of non-water-stable aggregates, aggregates that disintegrate in the first 30 s of water suspension and -circulation (Bieganowski et al., 2018; Yudina and Kuzyakov, 2023), was assessed for size classes <20 μm, 20–50 μm, 50–250 μm, and >250 μm. A summary of all aggregate stability parameters can be found in Table 2.

2.4. Statistical analysis

All statistical analyses were done using R (version 4.2.1) (R Core Team, 2020). The GHG fluxes, aggregate stability, and soil parameters were tested for normality by Shapiro-Wilk test and for homogeneity of variance by Levene's test. If necessary, normal distribution was achieved by log-transformation of the data. To assess the effect of the organic amendment application per location for all parameters, a paired *t*-test was performed. Treatment (organic-amended vs unamended) and practice (no-tillage vs tillage) effects, and differences between means were assessed using one-way ANOVA followed by Tukey's HSD *post-hoc* test for parameters with a normal distribution and homogeneous variance, or using Scheirer-Ray-Hare test followed by Dunn's *post-hoc* test for parameters without a normal distribution and/or homogeneous variance.

Individual linear regression analyses were performed to assess predicting variables of the CH_4, CO_2, and N_2O fluxes, and to asses organic matter content as a predicting variable of aggregate stability. All variables were not normally distributed (based on the Shapiro-Wilk test) and subsequently log-transformed. Linear models were tested for the entire dataset, and for subsets of organic-amended, unamended, no-tilled, and tilled plots, and for all six locations separately. Additionally, to determine controlling factors of GHG fluxes and of the soil aggregate stability, a redundancy analysis (RDA) was performed using the *vegan* package in R (Oksanen et al., 2022), using the variables treatment (organic-amended vs. unamended), practice (no-tillage vs. tillage), and all log-transformed soil and aggregate stability variables. The significance of aggregate stability and soil variables was tested by a Monte Carlo permutation test (999 unrestricted permutations).

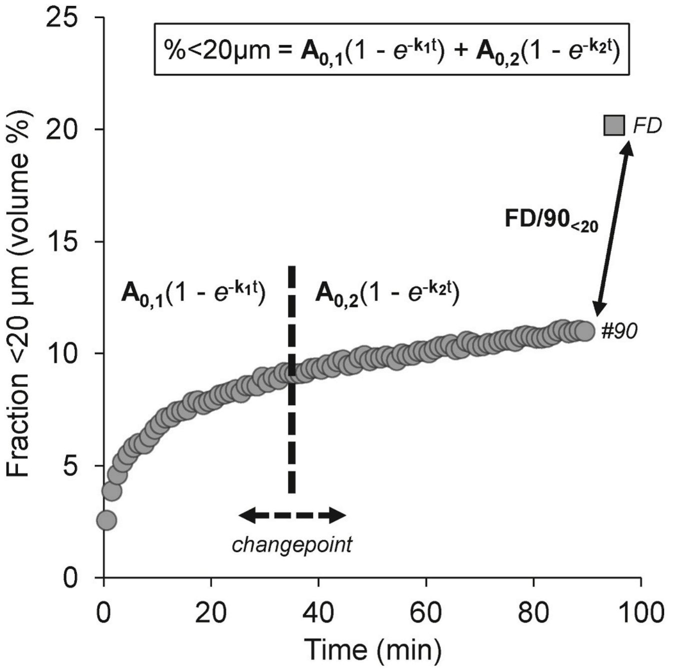

Fig. 1. Example of raw data output of the laser diffraction analysis for the fraction <20 μm for a sample of an unamended plot of location Wageningen. The changepoint dividing the data in two halves accommodating equation 1 is determined via a *changepoint* analysis. Subsequent curve-fit modelling provides parameters $A_{0,1}$, k_1, $A_{0,2}$, and k_2. The last measurement (#90) is made after 90 min, and the full dispersion (FD) is the average of five measurements after 5 min sonication after 90 min of measuring, together providing the FD/90 ratio for the <20 μm size class ($FD/90_{<20}$).

3. Results

3.1. Soil characteristics

The soil temperature, humidity, pore water electrical conductivity, and organic matter content of all soils are shown in Table S1. The full descriptive statistics are shown in Table S2 for pairwise comparison of the treatment effect (organic-amended vs. unamended), and the agricultural practice effect (tillage vs. no-tillage). At location Wageningen, the pore water conductivity was higher in organic-amended soils ($p \leq 0.01$), and at locations Melle and Zürich the organic matter content was higher in organic-amended soils than in unamended soils ($p \leq 0.01$ and $p \leq 0.001$, respectively) (Table S1), but there were no overall treatment effects on the soil characteristics (Table S2).

3.2. Greenhouse gas fluxes

All soils showed net CH_4 uptake (Fig. 2a). At location Wageningen, the organic-amended soil had a higher CH_4 uptake rate than the unamended soil ($p \leq 0.05$), but there were no overall treatment or practice

Table 2
Aggregate stability parameters.

Variable	Description	Interpretation	Generalized stability
1/DD	Ratio between the particle size distribution of the first measurement and the dry dispersion (DD). Indicates of the presence of non-water-stable aggregates, for size classes <20, 20–50, 50–250, and >250 μm.	$\lim_{1/DD \to 0^+ or \infty} \sim \uparrow$ non-water-stable aggregates $\lim_{1/DD \to 1} \sim \downarrow$ non-water-stable aggregates	$\lim_{1/DD \to 1} \sim \uparrow$ stability
k_1	Disintegration coefficient for the population of weak aggregates.	$\downarrow k_1 \sim \downarrow$ disintegration speed $\uparrow k_1 \sim \uparrow$ disintegration speed	$\downarrow k_1 \sim \uparrow$ stability
k_2	Disintegration coefficient for the population of stable aggregates.	$\downarrow k_2 \sim \downarrow$ disintegration speed $\uparrow k_2 \sim \uparrow$ disintegration speed	$\downarrow k_2 \sim \uparrow$ stability
$A_{0,1}/A_{0,2}$	Ratio between the populations of weak and stable aggregates.	$\downarrow A_{0,1}/A_{0,2} \sim \uparrow$ stable aggregates vs weak aggregates $\uparrow A_{0,1}/A_{0,2} \sim \uparrow$ weak aggregates vs stable aggregates	$\downarrow A_{0,1}/A_{0,2} \sim \uparrow$ stability
FD/90	Ratio between the full dispersion (FD) and the particle size distribution of the last measurement after 90 min. Indicates of the presence of persistent stable aggregates, for size classes <2, <20, 20–50, 50–250, and >250 μm.	$\lim_{FD/90 \to 0^+ or \infty} \sim \uparrow$ persistent stable aggregates $\lim_{FD/90 \to 1} \sim \downarrow$ persistent stable aggregates	$\lim_{FD/90 \to 0^+ or \infty} \sim \uparrow$ stability

effects (Table S2). Furthermore, there were no differences between CO_2 emission within locations (Fig. 2b), and there was no treatment effect. However, no-tilled soils did have a lower CO_2 emission than tilled soils ($p \leq 0.05$), and there was a significant interaction between treatment and practice ($p \leq 0.05$) (Table S2). Finally, all soils showed net N_2O emission, except the organic-amended tilled Vredepeel soil, where a limited N_2O uptake was measured (Fig. 2c). There were no differences between N_2O fluxes within locations, and also no overall treatment or practice effects (Table S2).

3.3. Soil aggregate stability

3.3.1. Dry dispersion size distribution

There was a clear difference between the dry dispersion of the soils between locations (Fig. S1). For the relatively clay-rich Lelystad, Melle, and Zürich soils (here defined as soils with clay % >7.5; Table 1) most of the material (70–80 vol %) was of size class >250 μm, whilst for the Vredepeel, Valthermond, and Wageningen soils the majority (50–60 vol %) was of size class 50–250 μm. In all soils, no particles of size <2 μm were measured in the dry dispersion, marking complete aggregation of clay particles into or within larger aggregates. Furthermore, particles of size <20 μm were less than 1.5 vol % of the total material in all soils, and for all soils in total 94–98 vol % of the material was of size >50 μm, emphasizing extensive aggregation of the smallest particles <50 μm (building units and small microaggregates) into larger aggregates. There were no differences between treatments within locations, and also no treatment or practice effects on the dry dispersion (Table S2).

3.3.2. Non-water-stable aggregates

The 1/DD, indicating the presence of non-water-stable aggregates (Table 2), showed clear differences, largely of the same trend as the dry dispersion. The soils with the majority of the material in size class >250 μm in the dry dispersion, had relatively high $1/D_{<20}$, $1/DD_{20\text{-}50}$ and $1/DD_{50\text{-}250}$, and low $1/DD_{>250}$ values (Fig. 3). This indicates that in these soils a large part of the material of size classes <20, 20–50, and 50–250 μm that is aggregated in the dry dispersion, is in fact part of non-water-stable aggregates of size >250 μm. Furthermore, the $1/DD_{20\text{-}50}$ showed that the organic-amended no-tilled Vredepeel soil had significantly less non-water-stable aggregates built up from the size fraction 20–50 μm than its unamended counterpart ($p \leq 0.05$). Melle's organic-amended soil had less non-water-stable aggregates of size >250 μm than the unamended soil, as shown by the $1/DD_{>250}$ ($p \leq 0.05$). But overall, there were no treatment or practice effects on the 1/DD for all size classes (Table S2).

3.3.3. Aggregate disintegration kinetics

The disintegration coefficient of the population of weak aggregates (k_1) ranged from 0.05 to 0.12 across all soils, being lowest in Valthermond and Wageningen (Fig. 4a). At Melle, the organic-amended soil had a significantly higher k_1 than the unamended soil ($p \leq 0.05$), but there was no overall treatment or practice effect on the k_1 (Table S2). The disintegration coefficient of the population of stable aggregates (k_2), which is typically a factor 10 smaller than the k_1 (Kasmerchak et al., 2019), varied from 0.004 to 0.008 between all soils (Fig. 4b). Valthermond soil had the highest k_2 values, and Wageningen soil had the lowest k_2 values. Melle's organic-amended soil had a significantly higher k_2 than the unamended soil ($p \leq 0.05$), but no overall treatment effect was observed. Notably, tilled soils had a significantly lower k_2 than no-tilled soils ($p \leq 0.05$) (Table S2). Additionally, the ratio of the population of weak aggregates versus the population of stable aggregates ($A_{0,1}/A_{0,2}$) varied between 0.29 and 0.67 (Fig. 4c). This showed that in all soils there were relatively more stable aggregates than weak aggregates, as the $A_{0,1}/A_{0,2}$ was <1.0. The $A_{0,1}/A_{0,2}$ was lowest for Vredepeel and Valthermond soils. For the tilled Vredepeel soil, the unamended soil had a significantly lower $A_{0,1}/A_{0,2}$ ($p \leq 0.05$), whereas for

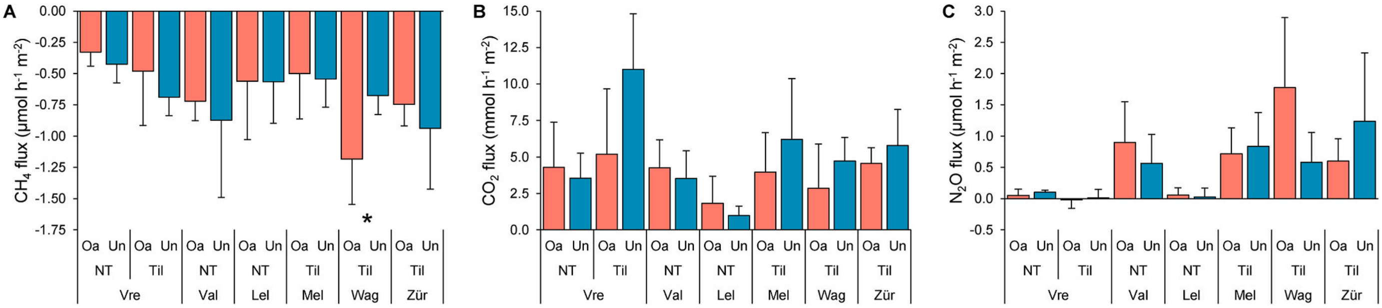

Fig. 2. Greenhouse gas fluxes (CH_4 (*A*), CO_2 (*B*), and N_2O (*C*); mean ± SD; $n = 4$–6) at visited field sites (Vre – Vredepeel; Val – Valthermond; Lel – Lelystad; Mel – Melle; Wag – Wageningen; Zür – Zürich) varying in agricultural practice (NT – no-tillage; Til – tillage) and treatment (Oa – organic-amended; Un – unamended). Significant differences are indicated with an asterisk (paired *t*-test; $p \leq 0.05$).

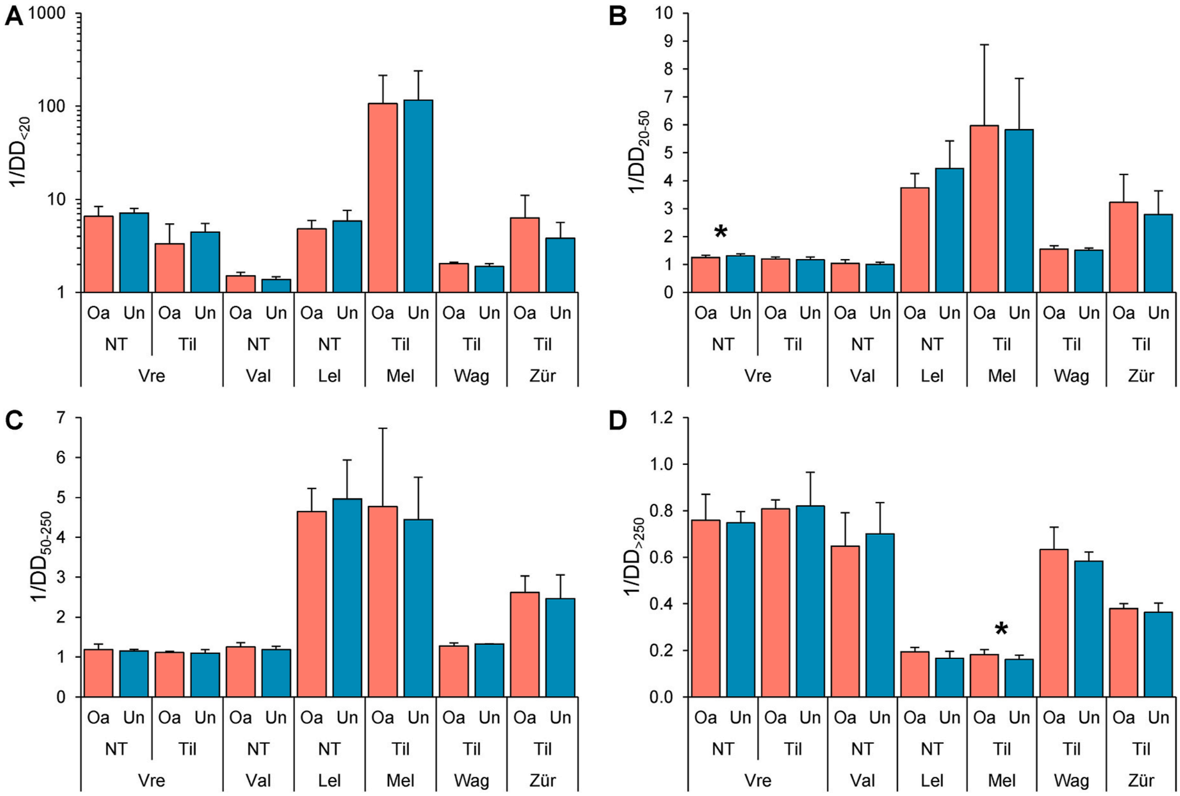

Fig. 3. The 1/DD ratio (mean ± SD; n = 4–6), indicating the presence of non-water-stable aggregates, for size classes <20 μm (*A*), 20–50 μm (*B*), 50–250 μm (*C*), and >250 μm (*D*) of soils of visited field sites (Vre – Vredepeel; Val – Valthermond; Lel – Lelystad; Mel – Melle; Wag – Wageningen; Zür – Zürich) varying in agricultural practice (NT – no-tillage; Til – tillage) and treatment (Oa – organic-amended; Un – unamended). Significant differences are indicated with an asterisk (paired *t*-test; $p \leq 0.05$).

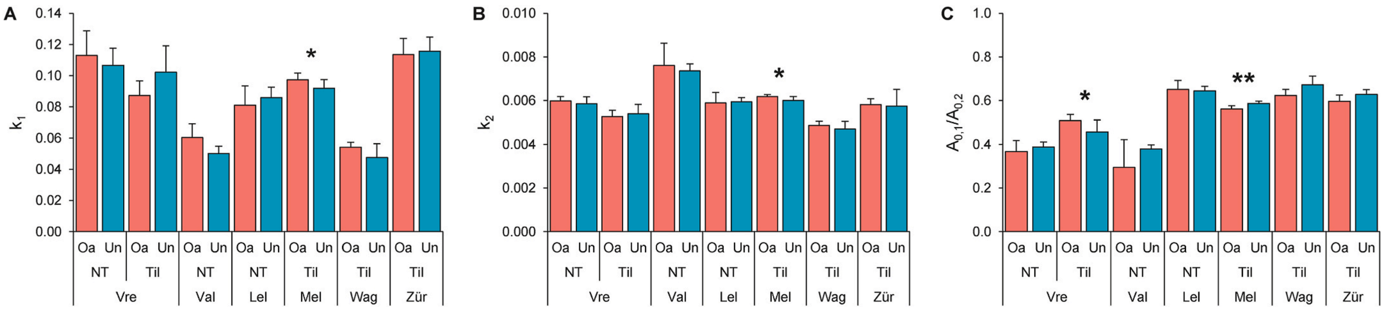

Fig. 4. The disintegration coefficient of weak aggregates k_1 (*A*), the disintegration coefficient of stable aggregates k_2 (*B*), and the ratio of weak versus stable aggregates $A_{0,1}/A_{0,2}$ (*C*) (mean ± SD; n = 4–6) of soils of visited field sites (Vre – Vredepeel; Val – Valthermond; Lel – Lelystad; Mel – Melle; Wag – Wageningen; Zür – Zürich) varying in agricultural practice (NT – no-tillage; Til – tillage) and treatment (Oa – organic-amended; Un – unamended). Significant differences are indicated with an asterisk (paired *t*-test; $^*p \leq 0.05$, $^{**}p \leq 0.01$).

the Melle soil the organic-amended soil had a significantly lower $A_{0,1}/A_{0,2}$ ($p \leq 0.01$). However, no overall treatment or practice effects were observed on the $A_{0,1}/A_{0,2}$ (Table S2).

3.3.4. Persistent stable aggregates

The FD/90 indicates the presence of persistent stable aggregates (Table 2). The $FD/90_{<2}$ and $FD/90_{<20}$ showed that in all soils there were persistent stable aggregates built up from material of size <2 μm (clay particles) and <20 μm (Fig. 5ab). Valthermond soil had notably high $FD/90_{<2}$ and $FD/90_{<20}$ values, pointing out a relatively high presence of persistent stable aggregates. Organic-amended Zürich soil had a significantly higher $FD/90_{<2}$ and $FD/90_{<20}$ than its unamended counterpart ($p \leq 0.01$ and $p \leq 0.001$, respectively), and Melle and tilled Vredepeel soil also had a significantly higher $FD/90_{<20}$ when comparing organic-amended versus unamended ($p \leq 0.05$ for both). This shows that in these organic-amended soils there was significantly more material of size <2 and < 20 μm part of persistent stable aggregates than in the unamended soil. However, no overall treatment or practice effects were found on the $FD/90_{<2}$ and $FD/90_{<20}$ (Table S2).

The $FD/90_{20\text{-}50}$ varied from 0.6 to 1.3 between all soils (Fig. 5c), indicating that there were substantial differences in the populations of persistent stable aggregates across the soils. Lelystad, Melle, Wageningen, and Zürich soils ($FD/90_{20\text{-}50}$ <1.0) had persistent stable microaggregates of size 20–50 μm, whilst Vredepeel and Valthermond soils ($FD/90_{20\text{-}50}$ >1.0) had persistent stable aggregates built up from material of size 20–50 μm. All soils except Lelystad had persistent stable large microaggregates of size 50–250 μm, as indicated by their $FD/90_{50\text{-}250}$ <1.0 (Fig. 5d). For Lelystad soil, no $FD/90_{>250}$ could be calculated as

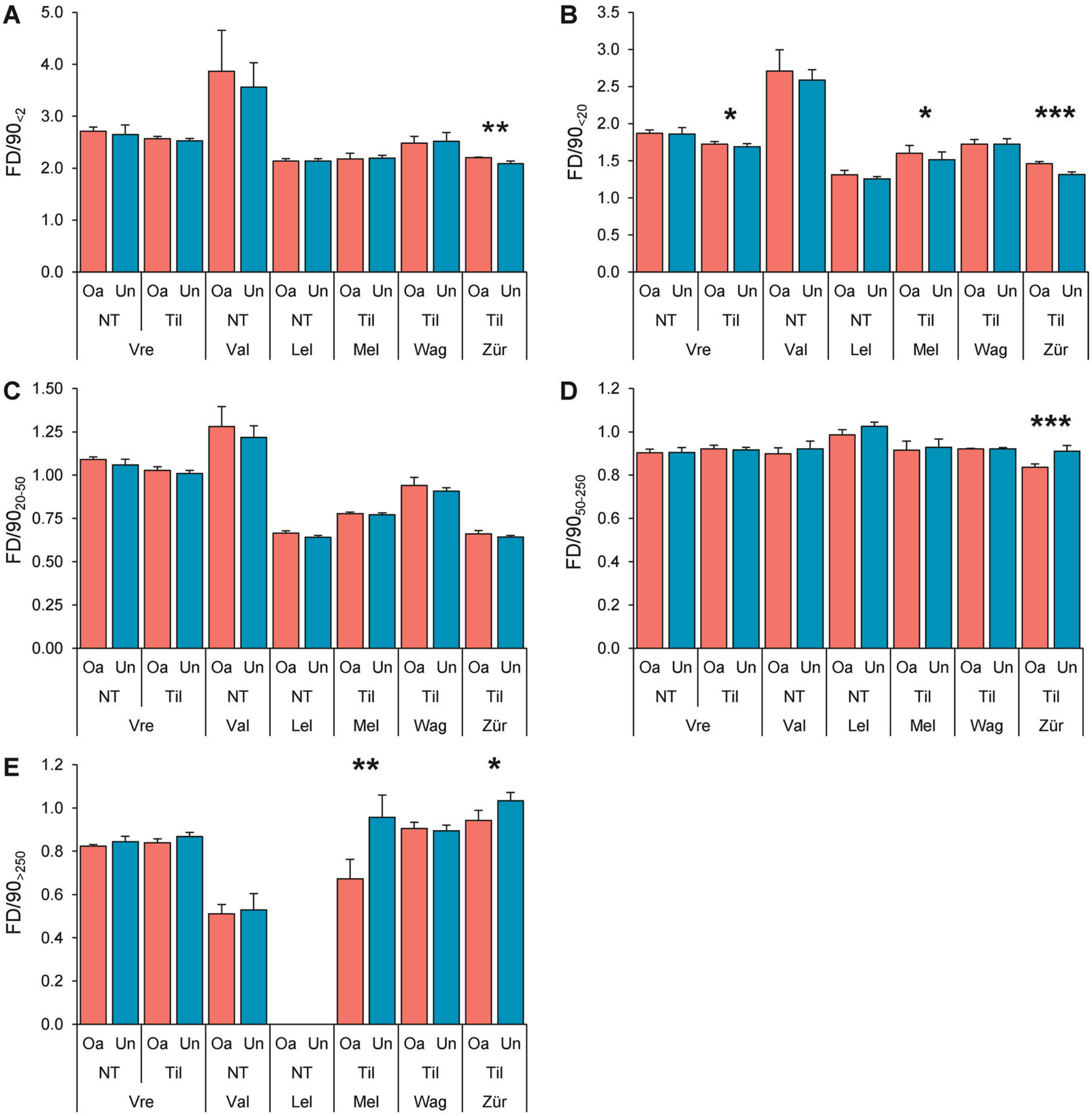

Fig. 5. The FD/90 ratio (mean ± SD; *n* = 4–6), indicating the presence of persistent stable aggregates, for size classes <2 μm (*A*), <20 μm (*B*), 20–50 μm (*C*), 50–250 μm (*D*), and >250 μm (*E*) of soils of visited field sites (Vre – Vredepeel; Val – Valthermond; Lel – Lelystad; Mel – Melle; Wag – Wageningen; Zür – Zürich) varying in agricultural practice (NT – no-tillage; Til – tillage) and treatment (Oa – organic-amended; Un – unamended). Significant differences are indicated with an asterisk (paired *t*-test; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$).

there was no material of size >250 μm present in the full dispersion, and no persistent stable aggregates of size >50 μm were present, as the $FD/90_{50\text{-}250} \approx 1.0$. There were significantly more persistent stable large microaggregates of size 50–250 μm in the organic-amended Zürich soil than in the unamended soil ($p \leq 0.001$). Overall, there were no treatment or practice effects on the $FD/90_{20\text{-}50}$ and $FD/90_{50\text{-}250}$ (Table S2).

The $FD/90_{>250}$ was <1.0 for most soils, indicating the presence of persistent stable small macroaggregates of size >250 μm (Fig. 5e). For Zürich soil, there were significantly more persistent stable small macroaggregates in the organic-amended soil ($p \leq 0.05$), whilst they were absent in the unamended soil, as the $FD/90_{>250} \approx 1.0$. Organic-amended Melle soil had significantly more persistent stable small macroaggregates than its unamended counterpart ($p \leq 0.01$). Also, there was an overall treatment effect on the $FD/90_{>250}$ ($p \leq 0.05$), signifying that organic-amended soils had more persistent stable small macroaggregates of size >250 μm than unamended soils. There was no practice effect on the $FD/90_{>250}$ (Table S2).

3.4. *Linear models of predicting variables of greenhouse gas fluxes*

Individual linear models were tested to predict the individual CH_4, CO_2, and N_2O fluxes from the soil and aggregate stability variables. All significant models are shown in Table 3, and the full results can be found in Table S3. The predictive value of soil or aggregate stability parameters for soil CH_4 uptake was limited, with R^2 values varying between 0.05 and 0.38 for all data and subsets. For the subset of unamended soils, no significant relations were found, but common predictors among the other datasets were the fractions <20 μm and 20–50 μm in the dry dispersion, and the pore water conductivity (showing positive and negative relationships, respectively) (Fig. S2). Interestingly, there was a significant negative relationship between k_1 and soil CH_4 uptake, found for organic-amended and no-tilled soils, suggesting that in these soils a higher disintegration rate of weak aggregates correlates with a lower soil CH_4 uptake rate, albeit with limited effect ($R^2 = 0.13$ and $R^2 = 0.18$, respectively) (Table 3 and Fig. S2). Furthermore, relatively strong

Table 3
Regression analysis results of the soil and aggregate stability parameters predicting greenhouse gas fluxes, and organic matter content predicting soil and aggregate stability properties for all samples and subsets (all variables log-transformed). Only significant models are shown, and the level of significance is indicated by an asterisk next to the adjusted R^2 ($*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$).

Samples	CH4 uptake					CO_2 emission					N_2O emission				
	Variable	Intercept	Slope	Std. error	Adj. R^2	Variable	Intercept	Slope	Std. error	Adj. R^2	Variable	Intercept	Slope	Std. error	Adj. R^2
All (n = 60)	DD < 20 μm	0.16	0.24	0.09	0.11 **	$FD/90_{50\text{-}250}$	3.81	−11.07	0.24	0.17 ***	*no significant relationships*				
	DD 20–50 μm	0.08	0.23	0.09	0.09 *										
	$1/DD_{<20}$	0.25	−0.04	0.09	0.05 *										
	Soil ECp	0.41	−0.10	0.09	0.10 *										
Organic-amended (n = 30)	DD < 20 μm	0.14	0.37	0.09	0.22 **	$FD/90_{50\text{-}250}$	3.86	−11.50	0.23	0.18 *	*no significant relationships*				
	DD 20–50 μm	0.00	0.36	0.09	0.18 *										
	k_1	0.35	−4.05	0.09	0.13 *										
	Soil ECp	0.57	−0.17	0.09	0.18 *										
Unamended (n = 30)	no significant relationships					$FD/90_{50\text{-}250}$	4.87	−14.50	0.23	0.26 **	*no significant relationships*				
No-tillage (n = 24)	DD < 20 μm	0.12	0.39	0.08	0.14 *	DD 50–250 μm	−0.37	0.58	0.21	0.33 **	DD 20–50 μm	−0.26	0.60	0.09	0.48 ***
	DD 20–50 μm	0.03	0.27	0.08	0.16 *	DD > 250 μm	2.22	−0.98	0.21	0.32 **	DD 50–250 μm	−0.25	0.22	0.12	0.16 *
	$1/DD_{<20}$	0.32	−0.20	0.08	0.22 *	$1/DD_{20\text{-}50}$	0.92	−0.82	0.21	0.32 **	DD > 250 μm	0.83	−0.43	0.11	0.25 **
	k_1	0.33	−4.24	0.08	0.18 *	$1/DD_{50\text{-}250}$	0.89	−0.71	0.21	0.30 **	$1/DD_{<20}$	0.34	−0.37	0.09	0.44 **
	$FD/90_{>250}$	0.49	−1.34	0.07	0.38 **	$1/DD_{>250}$	0.25	1.61	0.22	0.23 *	$1/DD_{20\text{-}50}$	0.24	−0.32	0.11	0.19 *
	Soil temp.	−0.92	0.97	0.08	0.14 *	$A_{0,1}/A_{0,2}$	1.02	−3.00	0.22	0.26 **	k_1	0.33	−7.15	0.11	0.29 **
						$FD/90_{<2}$	−0.45	1.71	0.22	0.25 **	k_2	−0.45	194.59	0.10	0.31 **
						$FD/90_{<20}$	−0.23	1.68	0.21	0.29 **	$A_{0,1}/A_{0,2}$	0.28	−1.18	0.12	0.15 *
						$FD/90_{20\text{-}50}$	−0.19	2.47	0.21	0.29 **	$FD/90_{<2}$	−0.51	1.04	0.10	0.41 ***
						$FD/90_{50\text{-}250}$	4.93	−15.24	0.18	0.50 ***	$FD/90_{<20}$	−0.35	0.97	0.10	0.41 ***
						OM content	0.09	0.56	0.24	0.13 *	$FD/90_{20\text{-}50}$	−0.23	1.09	0.11	0.23 *
											$FD/90_{50\text{-}250}$	1.45	−4.73	0.12	0.17 *
											$FD/90_{>250}$	0.56	−1.96	0.11	0.35 **
											OM content	−0.29	0.47	0.09	0.45 ***
											Soil temp.	−2.30	2.07	0.10	0.40 ***
											Soil ECp	0.59	−0.25	0.12	0.22 *
Tillage (n = 36)	DD < 20 μm	0.19	0.20	0.08	0.10 *	k_1	0.43	8.68	0.23	0.10 *	DD > 250 μm	0.71	0.51	0.17	0.12 *
	$1/DD_{<20}$	0.27	−0.05	0.08	0.10 *	$A_{0,1}/A_{0,2}$	1.77	−5.13	0.22	0.14 *	$1/DD_{>250}$	0.33	−0.81	0.17	0.10 *
	Soil temp.	0.64	−0.36	0.08	0.17 **						$A_{0,1}/A_{0,2}$	−0.59	4.01	0.16	0.16 **
	Soil ECp	0.45	−0.11	0.09	0.14 *						$FD/90_{20\text{-}50}$	0.69	−1.89	0.17	0.09 *
											OM content	1.04	−1.34	0.14	0.34 ***

negative relationships were found between the 1/DD$_{<20}$ and the FD/90$_{>250}$, and soil CH_4 uptake in no-tilled soils ($R^2 = 0.22$ and $R^2 = 0.38$, respectively) (Table 3). This indicates that in these no-tilled soils a relatively high presence of non-water-stable aggregates built up from material of size <20 μm, and a relatively low presence of persistent stable aggregates of size >250 μm, both correlate with a lower soil CH_4 uptake.

The R^2 values of predictive variables for the soil CO_2 emission ranged

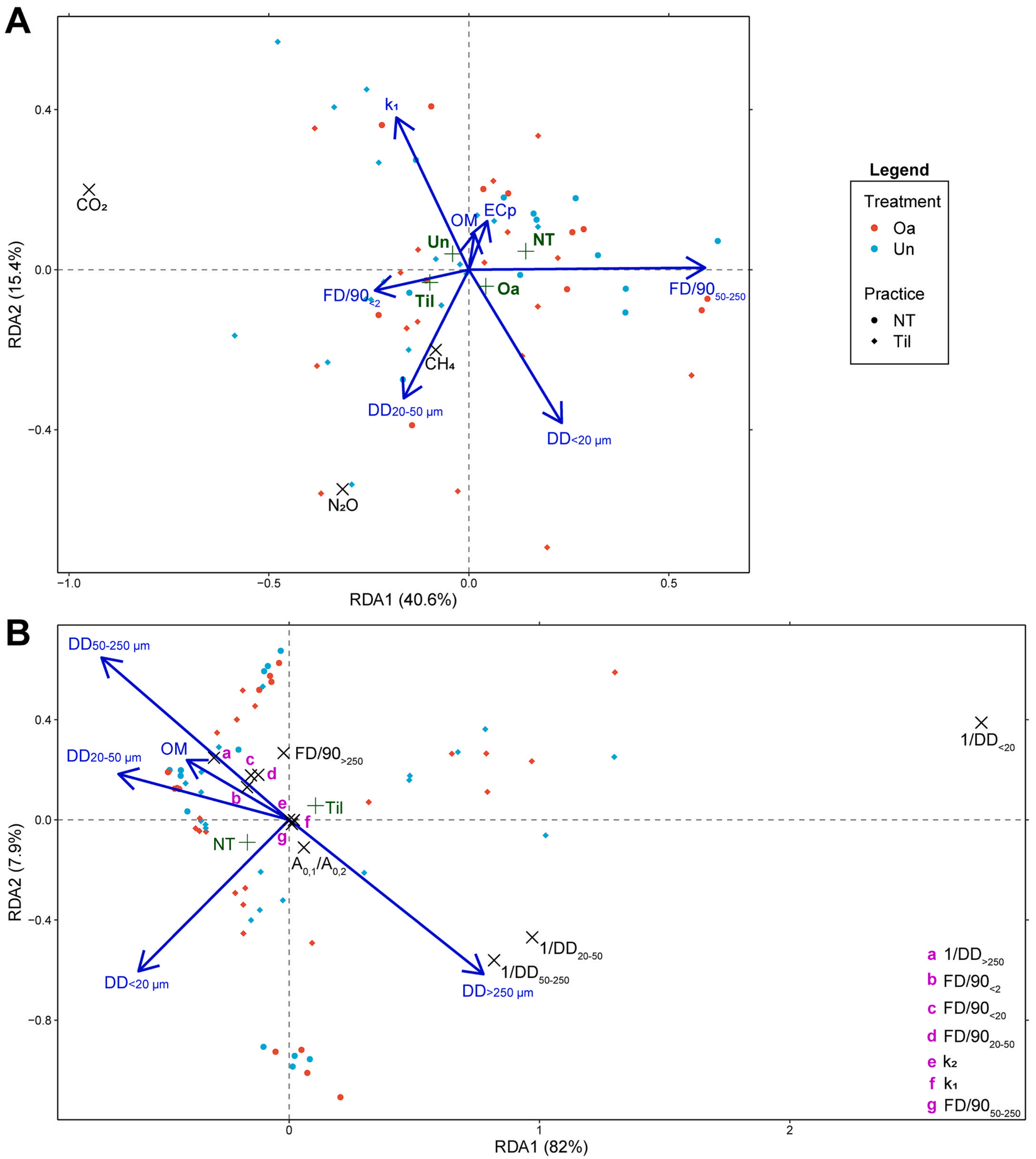

Fig. 6. RDA on variables explaining the variability observed in the soil GHG fluxes (*A*) and the soil aggregate stability (*B*), obtained using the soil parameters dry dispersion for all size classes (DD$_{<20\ \mu m}$; DD$_{20–50\ \mu m}$; DD$_{50–250\ \mu m}$; DD$_{>250\ \mu m}$), organic matter content (OM), and pore water conductivity (ECp); the aggregate stability parameters 1/DD for all size classes (1/DD$_{<20}$; 1/DD$_{20-50}$; 1/DD$_{50-250}$; 1/DD$_{>250}$), k_1, k_2, $A_{0,1}/A_{0,2}$, and the FD/90 for all size classes (FD/90$_{<2}$; FD/90$_{<20}$; FD/90$_{20-50}$; FD/90$_{50-250}$; FD/90$_{>250}$); and the management variables treatment (Un for unamended, Oa for organic-amended) and practice (NT for no-tillage, Til for tillage).

between 0.10 and 0.50 (Table 3). The $FD/90_{50\text{-}250}$ was the common significant, negative predictor, indicating that a relative lower occurrence of persistent stable aggregates of size 50–250 μm correlated with a low soil CO_2 emission (Table 3 and Fig. S3). For no-tilled soils, many aggregate stability variables were significant predictors, including 1/DD for 3 out of 4 size classes, $A_{0,1}/A_{0,2}$, and FD/90 for 4 out of 5 size classes. These relationships indicate that a higher generalized aggregate stability (Table 2) correlated with a higher soil CO_2 emission. The N_2O emission could not be predicted by the soil and aggregate variables for the complete dataset of all soils and subsets organic-amended and unamended soils. When dividing the data into subsets of no-tilled and tilled soils however, many variables proved to be significant predictors of soil N_2O emission (Table 3). Interestingly, for the variables $A_{0,1}/A_{0,2}$, $FD/90_{20-50}$, and organic matter content, contrasting relationships were found between no-tilled and tilled soils (Fig. S4). These predictors show that for no-tilled soils, a higher generalized aggregate stability and organic matter content correlated with a higher N_2O emission, whilst for tilled soils the opposite was true. Lastly, the organic matter content correlated significantly with the majority of the aggregate stability parameters (R^2 up to 0.91) (Table S4), showing a strong positive relationship with the generalized aggregate stability.

3.5. Controlling factors of greenhouse gas fluxes and soil aggregate stability

An RDA was performed to explore controlling factors of all soil GHG fluxes, showing that both the treatment and agricultural practice were well separated in separate quadrants (Fig. 6a). It resulted in RDA1 and RDA2 axes explaining 40.6% and 15.4% of the data variability, respectively. Significant correlations for the variables practice ($p \leq 0.001$), DD < 20 μm ($p \leq 0.01$), and k_1, $FD/90_{<2}$, $FD/90_{50\text{-}250}$ and pore water conductivity ($p \leq 0.05$), and moderate significant correlations for variables treatment, DD 20–50 μm, and organic matter content ($0.05 \leq p \leq 0.1$) were revealed by a permutation test. Furthermore, high soil CH_4 uptake and N_2O emission were positively associated with the $FD/90_{<2}$, DD < 20 μm and 20–50 μm, and negatively with k_1, organic matter content, and pore water conductivity. High soil CO_2 emission was positively associated with the k_1, $FD/90_{<2}$, and DD 20–50 μm, and negatively with the $FD/90_{50\text{-}250}$ and DD < 20 μm.

Additionally, another RDA was performed to explore controlling factors of the soil aggregate stability, resulting in RDA1 and RDA2 axes that explained 82.0% and 7.9% of the data variability, respectively (Fig. 6b). A permutation test revealed significant correlations for the variables practice, all dry dispersion size classes ($p \leq 0.001$), and organic matter content ($p \leq 0.01$). The RDA plot revealed that the organic matter content was positively associated with the $1/DD_{>250}$, $FD/90_{<2}$, $FD/90_{<20}$, and $FD/90_{20\text{-}50}$, and negatively with the $A_{0,1}/A_{0,2}$, $1/DD_{20\text{-}50}$, and $1/DD_{50\text{-}250}$, altogether indicating a strong positive relationship with the generalized soil aggregate stability (Fig. 6b and Table 2).

4. Discussion

4.1. The effect of sustainable agricultural management strategies on field GHG fluxes

In this study, the GHG fluxes and aggregate stability of agricultural soils under different sustainable agricultural management strategies were assessed and its governing factors were determined. All soils showed net CH_4 uptake, ranged between −1.18 and −0.33 μmol h^{-1} m^{-2} (Fig. 2a), indicating soil CH_4 uptake rates in the high end of the range reported by various studies on agricultural soil GHG fluxes (Table S5). In contrast to our expectation based on the use of organic amendments in laboratory experiments and the active indigenous methanotrophic bacterial community of composts (Brenzinger et al., 2021; Ho et al., 2015; van den Bergh et al., 2023), no effect of organic amendments on the CH_4 flux was found (Table S2 and Fig. 6a). However, this is in accordance with various other studies (Bayer et al., 2016; Krauss et al., 2017; Robertson et al., 2000; Skinner et al., 2019; Smith et al., 2011). It can be speculated that this is due to the spatial heterogeneity or distribution of organic amendments, the fluctuating nature of daily soil CH_4 fluxes and the environmental conditions in field situations, as for instance the cumulative daily precipitation prior to measuring (Flessa et al., 1995). Hence ideally, regular flux measurements should be performed to distinguish treatment effects from environmental and diurnal fluctuations (Collier et al., 2014; Drost, 2022; Yeboah et al., 2018). Furthermore, no effect of tillage practice on the CH_4 flux was found (Table S2), which agrees with previous studies (Regina and Alakukku, 2010; Ruis et al., 2022).

CO_2 emission ranged from 0.98 to 11.00 mmol h^{-1} m^{-2} (Fig. 2b), which is in line with previously reported CO_2 fluxes from agricultural soils (Table S5). There was no significant effect of the use of organic amendments, but no-tilled soils showed a significantly lower CO_2 emission than tilled soils (Table S2 and Fig. 6a), which agrees with the conclusions of other studies (Bayer et al., 2016; Ruis et al., 2022). This likely results from its impact on soil aggregation, as tillage breaks down soil aggregates, which typically protect soil organic matter from microbial decomposition (Ruis et al., 2022). Tilled soils showed a significantly higher disintegration coefficient of stable aggregates (k_2), confirming these relationships. Soil N_2O fluxes ranged from −0.02 to 1.78 μmol h^{-1} m^{-2} across all soils (Fig. 2c), falling well in the range of earlier reported N_2O fluxes from agricultural soils (Table S5). No amendment nor tillage effects were found on the N_2O fluxes, as also reported by various other studies (Bayer et al., 2016; Robertson et al., 2000; Ruis et al., 2022; Zhang et al., 2012). In addition, effects that have been found did not show a consistent trend (Afreh et al., 2018; Hansen et al., 1993; Meng et al., 2005; Skinner et al., 2019; Smith et al., 2011; Tellez-Rio et al., 2017), emphasizing the complex and variable nature of agricultural soil N_2O fluxes.

Overall, no conclusive effects of sustainable agricultural management strategies as the use of organic amendments and no-tillage practice were found on the CH_4 and N_2O fluxes of agricultural soils. But, the significant reduction in CO_2 emission in no-tilled soils highlights the potential of reduced intensity tillage management on atmospheric GHG mitigation.

4.2. Laser diffraction analysis as a method to asses soil aggregate stability

Assessing the aggregate stability of soils using LDA allows for a broad and quantitative perspective on the kinetics of aggregates of all size classes <2 mm (Fisher et al., 2017; Gyawali and Stewart, 2019). As larger aggregates disintegrate into smaller aggregates or individual mineral particles, this is reflected in a changing distribution of different size classes, revealing aggregate disintegration dynamics of weak and stable aggregates. Furthermore, LDA allows for the determination of the presence of both non-water-stable and persistent stable aggregates, and also their structural composition. It has been shown before that there is a high correlation between LDA and the traditional wet sieving methods (Gyawali and Stewart, 2019). Altogether, LDA proves to be an improved method for quantifying soil aggregate stability, as it can also overcome many of the limitations of the traditional wet sieving, like the lack of repeatability and the limited size range of measurable aggregates.

4.3. Sustainable agricultural management strategies improve the soil aggregate stability of agricultural soils

In all soils there was complete aggregation of the smallest fraction (clay particles <2 μm). Clay particles have a relatively large surface area and a negative surface charge, and thus aggregate more easily than electrical charge-less sand particles, which rely on charged organic matter for aggregation (Mitchell and Soga, 2005; Totsche et al., 2018). For all soils >94 % of the material was aggregated in particles of size >50 μm, and in the relatively clay-rich Lelystad, Melle, and Zürich soils the majority was aggregated in small macroaggregates of size >250 μm (Fig. S1). The 1/DD subsequently showed that in these soils, most of these macroaggregates were in fact non-water-stable (Fig. 3). Microaggregates with high intrinsic stability can be formed within non-water-stable aggregates and provide ecological functions within the soil matrix (Banwart et al., 2019; Rabbi et al., 2016; Six et al., 2000; Totsche et al., 2018; Wang et al., 2019a). Thus, despite their low stability, non-water-stable aggregates can be of substantial ecological relevance.

The disintegration coefficients of the two distinct populations of weak and stable aggregates (k_1 and k_2, respectively) of agricultural soils ranged between 0.05 and 0.12 (k_1), and between 0.004 and 0.008 (k_2) (Fig. 4). Kasmerchak et al. (2019) found k_1 and k_2 values a factor 3 to 5 and a factor 2 to 3 higher for forest and grassland soils, respectively. With decreasing management intensity (agriculture > grassland > forest), the disintegration coefficients increase, suggesting that less managed soils have a lower aggregate stability. However, the $A_{0,1}/A_{0,2}$ of the forest soils was >1.0 and thus substantially higher than the agricultural soils of this study, whilst the grassland soils had comparable $A_{0,1}/A_{0,2}$ values (Kasmerchak et al., 2019). This indicates that in the forest soils there are relatively more weak aggregates than stable aggregates, and in the agricultural and grassland soils, the opposite is true, implying better aggregate stability in managed soils compared to natural soils. Our results suggest that management practice, like agricultural tillage, leaves only the relative stable aggregates in soils. The relatively weak aggregates are already disintegrated or compacted, whilst in natural soils the loose structure is maintained (Or et al., 2021), which is confirmed by the significantly lower k_2 found in tilled agricultural soils compared to non-tilled soils (Table S2).

It is known that aggregates can persist in soils for decades (Totsche et al., 2018; Wilpiszeski et al., 2019), and are thus stable biogeochemical hotspots within the soil (Hartmann and Six, 2022; Or et al., 2021; Wang et al., 2019a). In this study, there were more persistent stable small macroaggregates in organic-amended than in unamended soils, as indicated by the $FD/90_{>250}$ (Table S2). This is also emphasized by the strong and significant correlations between the organic matter content of the soil and the $FD/90_{<2}$, $FD/90_{<20}$, $FD/90_{20\text{-}50}$, and $FD/90_{>250}$ (Fig. 6b and Table S4), which is in accordance with previous findings (Kasmerchak et al., 2019). More and stronger organo-mineral associations as a result of the addition of organic matter via organic amendments can result in higher aggregate stability (Totsche et al., 2018). Furthermore, the addition of organic matter introduces numerous binding agents to the soil matrix like a variety of soluble carbohydrates, thereby also increasing microbial biomass. Subsequently, more extracellular polysaccharides will be produced, serving as a binding agent and increasing inter-particle cohesion, hydrophobicity, and the overall aggregate stability (Abiven et al., 2009; Hernandez-Soriano et al., 2018). Interestingly, the soils with the longest organic application history (15+ years) and, consequently, significantly higher soil organic matter content following compost amendment (Melle and Zürich soils; Table 1 and Table S1), also had significantly more persistent stable aggregates (Fig. 5). We therefore provided evidence that long-term addition of organic amendments improves aggregate stability, confirming previous findings that associated the use of organic amendments with increased aggregation (Chavez-Rico et al., 2023; Mangalassery et al., 2019). Previously, it also has been shown that reduced intensity management can lead to higher aggregate stability via the improvement of the soil organic carbon content (Cooper et al., 2016; Ogle et al., 2005; Wang et al., 2022). Altogether, there is thus a great potential to improve the soil aggregate stability via sustainable management strategies.

4.4. Soil aggregate stability as a governing factor of soil GHG fluxes

The concept of aggregates as biogeochemical reactors is essential for understanding biogeochemical processes in soil, and correlative modelling is an insightful tool to this end (Wang et al., 2019a; Yudina and Kuzyakov, 2019). The linear models predicting soil GHG fluxes yielded R^2 values ranging between 0.05 and 0.50 (Table 3), and the ordination analysis could explain 55% of the variance of the soil GHG fluxes (Fig. 6a). The soil matrix and aggregates therein are a highly dynamic system (Mitchell and Soga, 2005; Totsche et al., 2018), and aggregate stability parameters can only have an indirect effect, but relevant nonetheless, on the biogeochemical processes of GHG emission and uptake (Banwart et al., 2019; Wang et al., 2019a).

The pore water conductivity and the size fractions <20 and 20–50 μm in the dry dispersion were the most common predictors of the soil CH_4 uptake (Table 3 and Fig. S2). A higher electrical conductivity has been shown to limit the methane uptake capacity in soil systems before (Gebert et al., 2003; Saari et al., 2004; Stegarescu et al., 2020). The availability of intracellular reducing equivalents like NADH, which serve as electron donors for the first step of the methane oxidation metabolic pathway (Chistoserdova et al., 2005; Khmelenina et al., 2018), is strongly decreased in conductive soils due to intracellular competition and consumption of these compounds, hampering CH_4 oxidation (Bodelier and Laanbroek, 2004; Semrau et al., 2010). Also, aggregate stability parameters k_1, $1/DD_{<20}$, and $FD/90_{>250}$ were found to be predictors of soil CH_4 uptake, especially for no-tilled soils (Table 3). Collectively, it was shown that an increased aggregate stability is correlating with an increased soil CH_4 uptake (Table 2), advocating for a governing relationship as proposed by Wang et al. (2019a). Improved aggregate stability allows for the establishment of anaerobic micro-spots within these stable aggregates as a result of the decomposition of occluded organic matter and subsequent increased microbial respiration (Angst et al., 2017; Bucka et al., 2019; Hoffland et al., 2020). CH_4 can be produced in these anaerobic micro-spots by methanogenic Archaea. The elevated CH_4 can activate MOB to co-oxidize atmospheric CH_4 (Bodelier et al., 2019; Wang et al., 2019b). It has been shown before that conventional MOB in soils can oxidize CH_4 to (sub-)atmospheric concentrations after exposure to high CH_4 concentrations (Cai et al., 2016; Ho et al., 2015, 2019). These conventional MOB can store energy derived from the oxidation of the high CH_4 concentration spikes in storage compounds like polyhydroxybutyrate (PHB) (Cai et al., 2016; Ho et al., 2013; Mason-Jones et al., 2022), which can be subsequently used to oxidize CH_4 at low concentrations. Concurrently, during the decomposition of organic matter, atmospheric trace gases such as hydrogen (H_2) and carbon monoxide (CO) are produced (Poissant et al., 2007; Schlegel, 1974). The oxidation of these atmospheric trace gases can serve as a supplemental energy source for bacteria (Bay et al., 2021; Cordero et al., 2019; Greening et al., 2015; Greening and Grinter, 2022), and MOB living solely off trace amounts of these gases have been described before (Tveit et al., 2019, 2021). It can thus be speculated that MOB can get the necessary energy to oxidize (sub-)atmospheric CH_4 by co-oxidation of other trace gases. The significant negative relationship between the k_1 and the soil CH_4 uptake for organic-amended and for no-tilled soils (Table 3 and Fig. 6a), suggests that sustainable agricultural management strategies can be used to improve soil aggregate stability. Thereby, the soil methane uptake capacity can be improved, contributing to global climate change mitigation.

For CO_2 emissions, the most common predictor was the presence of persistent stable aggregates of size 50–250 μm, whilst for no-tilled soils many aggregate stability parameters proved to be significant predictors (Table 3 and Fig. S3). Collectively it was indicated that a lower aggregate stability is associated with lower CO_2 emissions. This can be explained as the decomposition of organic matter mainly takes place within aggregates (Bucka et al., 2019; Or et al., 2021), and a lower aggregate stability will thus result in lower microbial decomposition rates and subsequent lower CO_2 production. Interestingly, soil N_2O fluxes could only be predicted by soil and aggregate stability parameters when subdividing the dataset into no-tilled and tilled soils (Table 3 and Fig. 6a). Moreover, in no-tilled soils a lower aggregate stability and organic matter content was significantly correlating with lower N_2O fluxes, whilst in tilled soils the opposite was true (Fig. S4), showing that tillage practice is an important steering factor of soil aggregate stability and N_2O fluxes. A relationship between the soil aggregate stability and N_2O fluxes as a result of the use of organic amendments was not found. Previous studies however, showed that larger aggregates contain more organic nitrogen (Liu et al., 2023), and that after organic fertilization both microbial denitrification gene abundance and N_2O emissions from large aggregates were increased (Højberg et al., 1994; Jiang et al., 2021; Lin et al., 2021; Yin et al., 2022), providing a probable explanation for the observed practice-affected relationships between soil aggregate stability and N_2O fluxes.

Altogether, we propose three gas migration scenarios under different environmental conditions related to the observed soil GHG fluxes and its relationship with aggregate stability. Firstly, in non-wet environmental conditions, organic carbon decomposition, microbial respiration, and denitrification rates can increase in more stable aggregates as described above. The subsequent potentially formed CO_2 and N_2O will diffuse through the soil pore network to the atmosphere. Concurrently, atmospheric CH_4 diffuses into the soil, and can be oxidized by MOB, making the soil a CH_4 sink. Secondly, in wet environmental conditions, the overall pore volume will be decreased as a result of an increased water-filled pore space, hampering gaseous diffusion in and out of the soil. Due to the increased soil moisture content, the anaerobic production of CO_2, CH_4 and N_2O will increase, especially in more stable aggregates because of the restricted diffusion of oxygen into the aggregates. Lastly, when wet and non-wet environmental conditions alternate, the increased CH_4 production in more stable aggregates during wet conditions, can stimulate the MOB in the aerobic outer zone of the aggregate to co-oxidize atmospheric CH_4 via the internal CH_4 production hypothesis, leading to increased soil CH_4 uptake during non-wet environmental conditions.

Both the organic amendment treatment and tillage practice were found to be significant governing factors of soil GHG fluxes (Fig. 6a). The interactions between soil aggregate stability and field GHG fluxes do however wager a potential balancing conflict when considering the desired effects on the total global warming potential of the soil. For instance, a lower aggregate stability seems to favor lower CO_2 and N_2O emissions for no-tilled soils, but a higher aggregate stability can concurrently lead to higher CH_4 uptake rates. Altogether, the results of this study show that soil aggregate stability is a governing factor of GHG fluxes in agricultural soils, and that sustainable agricultural management strategies could be used to steer soil aggregate stability and, both consequently and outright, GHG fluxes.

5. Conclusions

The determination of soil aggregate stability via laser diffraction analysis is a useful and improved method, which overcomes limitations of traditional wet sieving by, among others, allowing for the analysis of the continuous disintegration kinetics of various aggregate populations and size classes. Using this method, soil aggregate stability is found to be a governing factor of soil GHG fluxes. Sustainable agricultural management strategies can be used to steer the soil's aggregate stability and, both consequently and outright, soil GHG fluxes, thereby creating a potential to contribute to the mitigation of agricultural GHG emissions. Future research should focus on quantifying the overall effects of aggregate stability on the GHG balance in terms of global warming potential, and on determining the interactions between environmental conditions and the underlying mechanisms and associated microbes of the found relationships between aggregate stability and soil GHG fluxes.

CRediT authorship contribution statement

Stijn G. van den Bergh: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iris Chardon:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Márcio F.A. Leite:** Software, Methodology. **Gerard W. Korthals:** Resources, Funding acquisition. **Jochen Mayer:** Resources. **Mathias Cougnon:** Resources. **Dirk Reheul:** Resources. **Wietse de Boer:** Writing – review & editing, Supervision, Conceptualization. **Paul L.E. Bodelier:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109354.

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