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SHORT COMMUNICATION

Soil organic carbon and nitrogen in aggregates in response to over seven decades of farmyard manure application

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Abstract

The study aimed to evaluate the effects of long-term fertilisation on soil aggregation and the associated changes in soil organic carbon (SOC) and nitrogen (N) pools in aggregates. The combined application of mineral fertiliser and manure improved soil aggregation, SOC and N content in aggregates, compared to manure or mineral fertiliser alone, and thus proved to be a suitable fertilisation strategy to increase C sequestration in agroecosystems.

KEYWORDS
aggregates stability, agroecosystem, carbon sequestration, soil respiration

1 | INTRODUCTION

The stability of soil aggregates and associated carbon (C) is an important indicator for assessing overall soil health for better agroecosystem functions (Álvaro-Fuentes et al., 2009; Rieke et al., 2022). Soil aggregates protect soil organic C (SOC) from microbial decomposition by trapping it in aggregate fractions of different sizes (Goebel et al., 2009; Six et al., 2004). The efficiency of the aggregates in protecting the occluded SOC relies on their size, with macroaggregates (> 250 µm) being less efficient than microaggregates (Bossuyt et al., 2005; Six et al., 2004). Therefore, to maximise soil aggregation, it is essential to understand how land management practices such as fertilisation affect aggregate size distribution and associated SOC and nitrogen (N).

Fertilisation can generally increase soil aggregation as it directly affects above- and below-ground biomass production associated with high C input into the soil, for example, above-ground litter, root turnover and exudation, compared to unfertilised soils (Baumert et al., 2018; Řezáčová et al., 2021; Zhou et al., 2013). Within the fertilisation types, organic fertilisers are found to perform better than chemical ones alone in enhancing soil aggregation (Du et al., 2022; Wu et al., 2022; Zhou et al., 2013), mainly because of the fresh C input, which promotes microbial processes. Some studies reported that combining organic and synthetic mineral fertilisers is highly effective in improving soil C sequestration (Abdalla et al., 2022; Zhou et al., 2013). Such findings were mainly explained by the abundance of the important nutrients for microbial growth and activity from the mineral fertilisers in combination with a C source from organic fertilisers. However, the long-term combined fertilisation (organic + mineral fertiliser) effect on improving soil aggregation and the associated SOC and N content.

The present study used soil samples from selected fertilisation practices, mineral fertiliser (+s), only manure (+m), both fertiliser types (+s+m) or no fertiliser over seven decades (after 1942) from the experimental site Dikopshof (North Rhine-Westphalia, Germany). The study aimed at evaluating the effects of the three fertilisation practices on the aggregate size distribution and the associated SOC and N content in connection to soil basal respiration. Our previous study from the same experimental plots showed that the combination of manure and mineral fertiliser (+s+m) yields a higher net SOC gain, which was explained by the lower priming effects, compared to mineral fertiliser or unfertilised soil (Abdalla et al., 2022). Here, we aim to test whether the increased SOC content in combined fertilised soils can further be explained by the higher stability of soil aggregates and aggregate-associated SOC and N, compared to other fertilisation practices, in addition to lower priming effects.

2 | MATERIALS AND METHODS

2.1 | Study site

The experiment was located at the Dikopshof research farm, Germany (50°48′ 21″ N: 6°59′ 9″ E: 62 m asl), established in 1904. The soil is classified as a Haplic Luvisol derived from loess covering a sand layer. The silty loam topsoil is a ploughed Ap horizon (0–30 cm), followed by an illuvial Bt horizon (30–80 cm), Bw horizon (80–100 cm) and C horizon (100–160 cm). Subsequent sand and gravel layers occur beyond 160 cm depth (Holthusen et al., 2012). Soil bulk density increased from about 1.4 g cm^{-3} in the topsoil to about 1.5–1.6 g cm^{-3} below 30 cm depth. The topsoil has a mean pH ($CaCl_2$) of 6.7 (Seidel et al., 2021). The climate has a mean (1906–2014) annual temperature and precipitation of 10°C and 630 mm, respectively (Hadir et al., 2020). The experimental site has long been subjected to a 5-year crop rotation consisting of sugar beet, winter wheat, winter rye, potato/oat (potato was replaced by oat in 1953) and a fodder legume crop, initially red clover, replaced by alfalfa and Persian clover after 1967 (Seidel et al., 2021).

2.2 | Experimental set-up

The experiment comprises six basic fertilisation practices: NPKCa, PKCa, NKCa, NPCa, NPK and unfertilised control (N, nitrogen; P, phosphorus; K, potassium; and Ca; calcium). These basic practices were either combined with farmyard manure (+m), further additional mineral fertiliser (+s) supplementing the nutrients applied via manure (e.g., NPKCa+m and NPKCa+s receive the same amount of nutrients), both (+s+m) or no further fertiliser (Seidel et al., 2021), leading to a total of 24 treatments. No significant change occurred to the manure fertilised plots (+m and +s+m) from 1904 to the sampling date in 2019, but the +s and the unfertilised plots received ≈ 2.5 tonnes of dry matter manure ha^{-1} $year^{-1}$ before 1942. The current study considered four treatments (same as Abdalla et al., 2022): fertilised with mineral fertiliser in a similar amount of nutrients as compared to the manure application (+s), with cattle farmyard manure (+m), with mineral fertiliser and with manure (+s+m), and unfertilised control (uf; Table 1). There was no change in the four fertilisation practices after 1942 to the sampling date in 2019, thus maintaining the practices over the last 77 years.

Each plot has a 15 × 18.5 m total size, with a core experimental plot of 9 × 10 m. The farmyard manure application rate was 20 t ha^{-1} $year^{-1}$ fresh manure (25% dry matter content and a C:N ratio of 25:1), applied to sugar beet, winter rye, potato, that is, 60 t ha^{-1} per crop rotation (Hadir et al., 2020). The field has been ploughed (≈ 30 cm) yearly, and the crop residues were regularly removed (but not the potato leaves).

2.3 | Soil sampling, aggregates fractionation and analysis

The soil samples used for the present study were collected in July 2019 from the upper 20 cm layer, with an initial SOC values of 5.7 ± 0.1, 6.2 ± 0.3, 8.4 ± 0.2 and 9.5 ± 0.3 g kg^{-1} soil for uf, +s, +m and +s+m, respectively (Abdalla et al. 2022). The present study used a one-step wet-sieving aggregate fractionation method of air-dried and sieved (through 8 mm) soil samples. Three aggregate fractions, macroaggregate (> 250 μm), microaggregate (63–250 μm) and silt/clay-sized fractions (< 63 μm), were obtained using the wet-sieving method (Six et al., 1998). Briefly, a subsample equivalent to 100 g dry soil was submerged in 100 mL deionised water for 5 min and poured into a 250-μm sieve placed inside a dishpan with deionised water (≈ 10 cm depth). The aggregate separation was done manually by moving the sieve up and down (50 times) in the deionised water. The materials that remained on the top of the sieve represent the macroaggregates collected by backwashing the sieve into a pre-weighted aluminium pan. The suspension that passed through the 250-μm sieve was poured into a 63-μm sieve, and the above-wet sieving procedure was repeated to separate the microaggregate and silt/clay-sized fractions. The separated fractions plus the water were oven-dried at 60°C until constant weight. The size distribution of each aggregate fraction was calculated as a percentage of the dry soil, and the mean weight diameter (MWD) was calculated using the equation by Kemper and Rosenau (1986):

$$MWD = \sum_{i=1}^{n} XiWi \,, \quad (1)$$

where Xi is the mean diameter for each fraction size, Wi is the proportional weight of the fraction from the total dry weight of soil, and n is the number of aggregate classes.

The soil C and N content in the aggregate fractions were analysed using Elemental Analyser (EA3100, EuroVector, Tecnologico di Pavia). The total soil C was considered equal to SOC due to the lack of reaction with 1 M HCl. The recovery of the weight, SOC and N content of all combined aggregate size fractions in relation to the bulk soils ranged from 98% to 100%.

2.4 | Basal respiration

Basal CO_2 efflux was measured from the composite samples (16 samples) used for the aggregate fractionation over 33 days in controlled

TABLE 1 Application rate per element (kg ha^{-1} year^{-1}) for each treatment (i.e., unfertilised control (uf), mineral fertiliser (+s), cattle farmyard manure (+m), and a combination of both mineral and manure fertiliser (+s+m), by crop and fertilisation sources at the Dikopshof experimental site.

		N-P-K-Ca applied (kg ha^{-1} year^{-1})				
Treatments	**Fertilisers**	**Winter wheat**	**Winter rye**	**Sugar beet**	**Potato**	**Persian clover**
Unfertilised control (uf)	No fertiliser	0–0–0–0	0–0–0–0	0–0–0–0	0–0–0–0	0–0–0–0
Mineral fertiliser (+s)	Base mineral	0–0–0–0	40–22–83–0	40–22–82–0	40–22–83–0	0–0–0–0
Manure (+m)	Manure	0–0–0–0	40–22–83–0	40–22–82–0	40–22–83–0	0–0–0–0
NPKCa+s+m (+s+m)	Base mineral	120–31–116–0	40–31–116–0	80–31–116–0	50–31–116–0	0–31–116–1143
	Manure	0–0–0–0	40–22–83–50	40–22–83–50	40–22–83–50	0–0–0–0
	Additional mineral	0–0–0–0	40–22–83–0	40–22–83–0	40–22–83–0	0–0–0–0

conditions incubation using a CO_2 isotope analyser (CCIA–38d-EP, Los Gatos Research (LGR) (Abdalla et al., 2022). In the current study, the cumulative basal respiration over the incubation period was used to further investigate its relationships to the aggregate stability and SOC and N associated with the aggregate fractions.

2.5 | Statistical analysis

Due to the lack of true replication at the experimental site, four demarcated subplots in each plot served as pseudo-replicates (Gomez & Gomez, 1984). While using such an approach is not ideal, it is justifiable considering the value of the long-term (over seven decades) experiment site and possible statistical solutions (Davies & Gray, 2015; Liebig et al., 2013). Therefore, the aggregate size distribution, SOC, N and C:N within the aggregate fractions were statistically analysed using a linear mixed model with the fertilisation practices considered as fixed effects and the pseudo-replicates as random effects. Means were compared using Holm–Sidak (at $p \leq 0.05$), a recommended powerful test for pairwise comparisons (Holm, 1979). The Kaiser–Meyer–Olkin was 0.67, and Bartlett's sphericity was significant ($p < 0.001$) indicating the data suitability for principal component analysis (PCA). Therefore, PCA based on a correlation matrix was used to further investigate the effect of aggregate size, SOC and N within the aggregates on the soil basal respiration. SigmaPlot software (14.5, Systat Software Inc., 2013) was used for the statistical analysis.

3 | RESULTS

3.1 | Aggregates stability and size distribution

The fertilisation regime had a significant influence on the MWD: The combined mineral and manure fertilisation (+s+m) showed the highest aggregate stability, 28% higher than the unfertilised control (uf) and 8% higher than manure (+m) or mineral fertiliser alone (+s) (Figure 1). However, this was not the case for the basal respiration rates measured for the same samples, where +m and +s+m induced statistically similar basal respiration rates. The greatest cumulative basal respiration was observed in +m and +s+m on one side, and the lowest was recorded in +s and the unfertilised (uf) on the other site. Following the MWD, the aggregate size distribution also differed significantly between fertilisation types, with uf having the largest proportion of the silt/clay-sized fraction (<63 μm), followed by +s and smallest proportion of +s+m (Figure 2). Oppositely, the microaggregates proportion to total soil increased in the following order: uf < +s < +m < +s+m. However, a significant difference was observed between uf and +s on the one hand, and +m and +m+s on the other hand. Against our expectations, no differences were found between the macroaggregate fractions of the fertilisation practices.

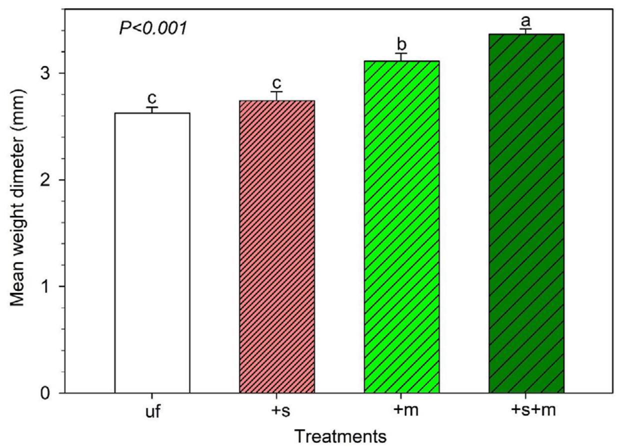

FIGURE 1 Mean ± standard error ($n = 4$) of the mean weight diameter (MWD) from the fertilisation types (uf, unfertilized control, mineral fertiliser (+s), manure (+m) and mineral fertiliser and manure (+s+m). Means followed by different letters indicate a significant difference between the fertilisation types at $p \leq 0.05$.

3.2 | SOC and N in the aggregates fractions

The SOC associated with the aggregate fractions was significantly lower in the uf and +s fertiliser applications than in manure amendments (i.e., +m and +s+m; Figure 2B). Interestingly, significant differences in SOC between +m and +s+m treatments were only observed in the macroaggregate fractions, where SOC content was 22% greater in +s+m than +m (Figure 2B). Similarly, N content associated with

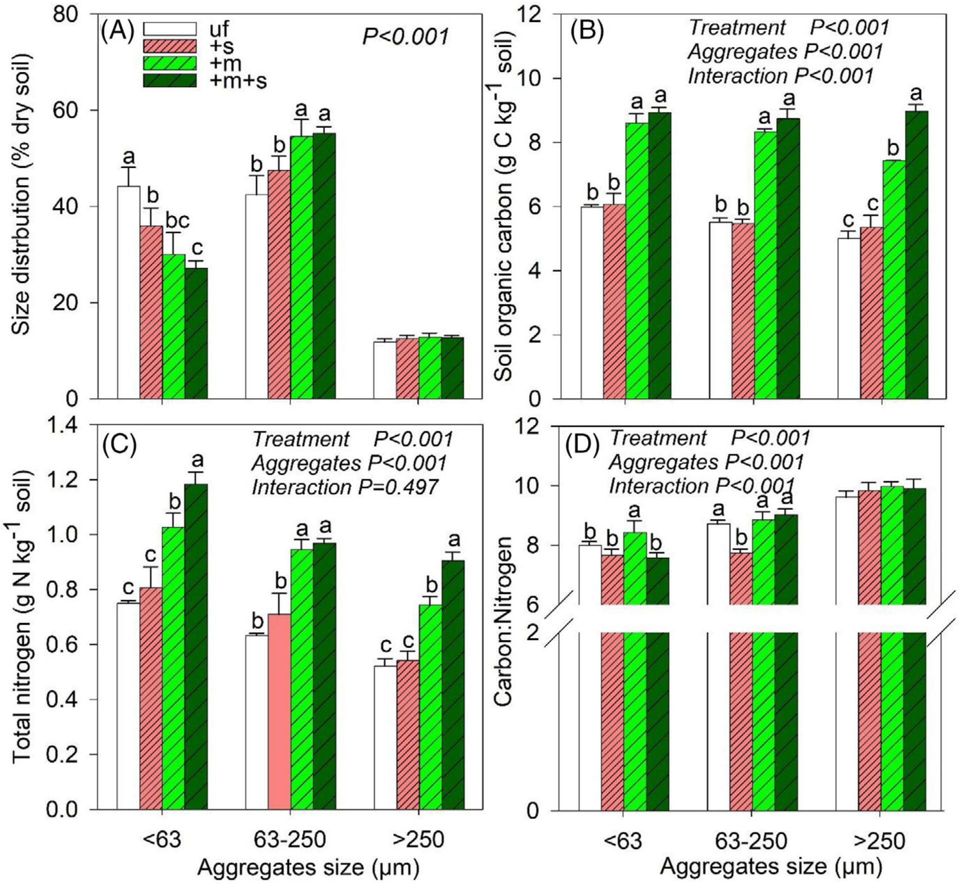

FIGURE 2 Mean ± standard error ($n = 4$) of the (A) aggregate size distribution, organic carbon concentration (B), and nitrogen (N) concentration (C) and (D) carbon: N ratio from the fertilisation types (uf, unfertilised control, mineral fertiliser (+s), manure (+m), and mineral fertiliser and manure (+s+m). Means followed by different letters within the aggregate fraction indicate a significant difference between the fertilisation types at $p \leq 0.05$.

the aggregates fractions was greater in soils with manure application than +s and uf (Figure 2C). The N content within the silt/clay-sized fraction increased from uf to +s+m, with uf and +s inducing similar N content in all aggregate fractions. Among the fertilisers with manure application, +s+m had a greater N content within the silt/clay-sized and macroaggregate fractions than +m, but it was similar within the macroaggregate fractions. The unexpectedly similar SOC and N associated with silt/clay-sized to the other fractions (Figure 2B,C) may have resulted from using a one-step wet sieving method, which needs to be considered when interpreting the outcome of this study. The C:N ratio within the aggregates fractions varied less between the fertilisation types than SOC and N content, with +m being highest in the silt/clay-sized fraction and +s lowest in the microaggregate fraction (Figure 2D).

4 | DISCUSSION

Long-term manure application significantly increased soil aggregate stability, with the combined mineral and manure fertiliser (+s+m) being greater than manure alone (+m; Figure 1). The increased soil aggregation under organic manure applications (either alone or combined with mineral fertiliser), compared to the unfertilised and chemically fertilised plots, is obviously due to the continuous fresh C inputs from manure. The continuous fresh C inputs increase the biological binding agents and thus promote soil aggregation and the associated SOC and N (Guo et al., 2019; Six et al., 2004; Zhang et al., 2014). However, the reason for the greater aggregation, SOC and N accumulation in +m+s than manure only (+m) could be the rhizodeposition and root litter input associated with the enhanced biomass production as a result of the highest amount of fertilisers applied in +m+s in the study site (Hadir et al., 2020; Rueda-Ayala et al., 2018). Hadir et al. (2020) reported an increase in the shoot, root growth and the overall dry matter of sugar beet subjected to +s+m, compared to other treatments. Rueda-Ayala et al. (2018) found that +s+m developed high-yield trends for winter wheat, winter rye, sugar beet and potato fresh matter yields using linear regression of yield data recorded from 1953 to 2009 at the experimental site. Thus, implying greater root and rhizodeposition input in +s+m than +m, which has a well-known contribution to the soil SOC and N accumulation and aggregate formation (Baumert et al., 2018; Villarino et al., 2021). Therefore, the fresh C inputs by manure and enhanced root litter and rhizodeposition increase soil microbial

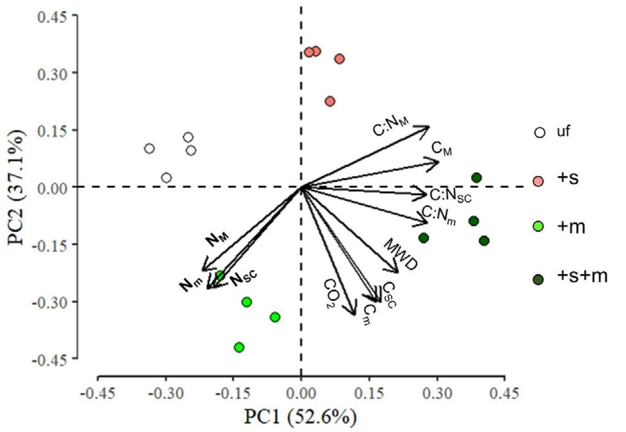

FIGURE 3 Principal components (PCs) bi-plot showing the relations between soil basal respiration (CO_2) and aggregate associate parameters (MWD, mean weight diameter; C, organic carbon concentration; N, nitrogen content and; C: N, carbon to nitrogen ratio in the different aggregate fractions (SC, silt/clay sized; m, microaggregates and; M, macroaggregate fractions) from the fertilisation practices (uf, unfertilised control, mineral fertiliser [+s], manure [+m], and mineral fertiliser and manure [+s+m]).

biomass and activity, which in turn facilitated SOC stabilisation within the aggregates (Paterson et al., 2007; Řezáčová et al., 2021).

The combined application of manure and chemical fertiliser also increased SOC and N content associated with all aggregate fractions, compared to +m (Figure 2B,C), indicating that the mineral fertiliser is more beneficial for SOC sequestration when applied in combination with manure. Such findings were explained by the lower priming effect causing a reduction in SOC decomposition rate in +s+m due to the abundance of nutrients, mainly dissolved N, compared to +m (Abdalla et al., 2022; Essel et al., 2021). The higher soil N abundance limits the soil microbial need for soil organic matter mining for nutrients, thus reducing the decomposition rate and promoting C stabilisation (Chen et al., 2022). In addition, the greater final SOC content in +s+m than +m observed in the current study site can further be explained by the physical protection of SOC within the aggregates as indicated by the greater MWD observed in the present study (Figure 1).

In fact, the physical and chemical stabilisation of the SOC within the aggregates might have a larger impact on the SOC sequestration, compared to other mechanisms such as the priming effect (Kan et al., 2022). This is mainly because the aggregates form a barrier that restricts microorganisms accessibility to the occluded organic substrates (Six et al., 2000; Zheng et al., 2018). However, enhanced soil aggregation does not always mean a lower organic matter decomposition rate and consequently less soil respiration. As shown in the present study by the PCA (Figure 3; where the first two PCs explained 89.7% of the total variation), the basal soil CO_2 correlated positively with the MWD and the SOC within the macroaggregate and silt/sized fractions, indicating a simultaneous increase of soil aggregation and soil basal respiration, probably due to the enhanced microbial biomass and activity harnessed by manure application. Manure was found to increase total microbial biomass, bacterial and fungal diversity and activities inducing high basal respiration (Francioli et al., 2016; Řezáčová et al., 2021). In general, the combined application of manure and mineral fertiliser promoted soil C stabilisation by both mechanisms, reduced priming as well as enhanced aggregation, supporting the overall hypothesis of the current study.

5 | CONCLUSION

Overall, the combination of organic and mineral fertilisers proved to be a better fertilisation practice for enhancing soil aggregation, leading to a higher potential of agroecosystem for C sequestration than individually applying mineral fertilisers or manure. This was explained not only by the direct effect of organic manure application but also by microbial essential nutrients (N, P and K) supplied from the mineral fertiliser, which promotes aggregation and makes the soil microbes preferentially consume the easily available nutrients instead of mineralising soil organic matter.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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