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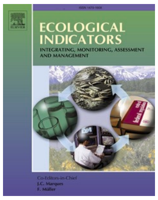

Original Articles

Effects of decadal nitrogen addition on carbon and nitrogen stocks in different organic matter fractions of typical steppe soils

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ABSTRACT

Recent frameworks have proposed that division of soil organic matter (SOM) into particulate and mineral-associated organic matter (POM and MAOM) can help us better understand SOM cycling and its responses to increasing atmospheric nitrogen (N) deposition. However, responses of these factions to N deposition with combination of their relative distribution across soil profile remain unclear. Here we determined total N and soil organic carbon (SOC) as POM and MAOM separately in soils at depths of 0–10, 30–40 and 70–100 cm after 10-year N addition (at rates of 50, 10, 2 and 0 g m^{-2} yr^{-1}) in a typical steppe. We further calculated their stocks in POM, MAOM and bulk soil and detected their relationships with both physicochemical features and microbial properties. Nitrogen addition increased the stocks of SOC (POM: +23 %; MAOM: +11 %) and total N (POM: +27 %; MAOM: +10 %) in both POM and MAOM fractions in topsoil (0–10 cm), but increased only in MAOM in 30–40 cm (SOC: +24 %; total N: +24 %) and 70–100 cm (SOC: +15 %; total N: +13 %) soils. Moreover, the increasing effects were strengthened with increasing N addition rates. We found that the share of SOC and total N in the MAOM was slightly decreased by N addition in topsoil, but significantly increased in deeper soils. Soil physicochemical features exerted stronger controls than microbial properties in the distribution of SOC and total N in the two fractions regardless of soil depth. SOC and total N contents of MAOM were correlated negatively with soil pH across the soil profile, and were correlated positively with bulk soil total N, dissolved organic N and inorganic N. Our findings imply that more soil C would be stabilized as MAOM under increasing atmospheric N deposition, and therefore the C saturation level of MAOM should be a target for further studies and be considered in predicting SOM dynamics, especially in *N*-limited grassland ecosystems.

1. Introduction

Soil organic matter (SOM) is the Earth's largest carbon (C) pool, which plays an critical role in both global C cycle and ecosystem functioning (Schmidt et al., 2011; Bossio et al., 2020). Unfortunately, the mechanisms of its degradation and stabilization are so complex that we could not accurately monitor and predict its dynamics under global changes, such as atmospheric nitrogen (N) deposition (Amundson and Biardeau 2018; Wiesmeier et al., 2019; Bossio et al., 2020). Recent studies have suggested that the division of SOM by size into two parts, i.e., particulate organic matter (POM) and mineral-associated organic matter (MAOM), is an effective way to describe SOM behavior more clearly because the overall response of SOM will depend on the two fractions (Sokol et al., 2019; Lavallee et al., 2020; Rocci et al., 2021; Lethold et al., 2022). POM is mainly comprised of partly decomposed fragmented plant litter and is therefore considered as an easily

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accessible part of SOM for microorganisms due to its residence in gaps among larger aggregates. On the contrary, MAOM is mainly comprised of small molecular weight compounds (*e.g.,* microbial polysaccharides and amino sugars) and is thought to be a recalcitrant part of SOM because of its being strongly bonded with surfaces of minerals and thus being difficult to be attacked by microorganisms (Lavallee et al., 2020).

Considering that atmospheric N deposition is a crucial driving factor of global changes and its potential impacts on SOM, previous studies have investigated N addition effects on C and N stocks (He et al., 2014; Wang et al., 2019; Xu et al., 2021). Importantly, response of bulk SOM to N deposition can not represent that of individual soil fractions (i.e., POM and MAOM). Even with no change of bulk SOM under N addition, portions of POM or MAOM to total SOM may also be altered because of the transformation processes between POM and MAOM by either a physical transfer path (sorption and desorption processes) or a microbial path (Averill and Waring 2018; Robertson et al., 2019). A latest meta-analysis showed that N addition increased SOC content not only in bulk soil, but also in both POM and MAOM fractions, and addressed that soil depth and N treatment duration were important moderators (Rocci et al., 2021). A field experiment indicated that soil POM is possibly more vulnerable to N addition than MAOM in an alpine meadow ecosystem (Chen et al., 2021a). These inconsistent results highlight the need for more detailed work to identify the responses of individual soil fractions to future increasing atmospheric N deposition. For instance, most previous studies did not show detailed data on the mass of soil fractions, and not to mention the C and N stocks in soil fractions under condition of N addition (Averill and Waring 2018; Lugato et al., 2021). In addition, most previous studies focused only on the top soil even though the deep soil (20 ~ 100 cm soil) could store more than 50 % SOM and may thus play a crucial role in mitigating global climate change (Schmidt et al., 2011; Angst et al., 2018).

Some studies have aimed to identify the stabilization mechanisms of POM and MAOM by theoretical analysis and experimental measurement, but until present, most of them still remain at a theoretical stage (Lal 2018; Robertson et al., 2019; Adamczyk 2021; Rocci et al., 2021). Insufficient field experimental data is an important cause for not being able to understand these mechanisms (Rocci et al., 2021). Increasing plant biomass means more plant-originated C and N inputs to soil from litter decomposition although the final distribution of C and N in the two fractions depends on soil physicochemical features and soil microbial properties (Muhammad et al., 2017; Mikutta et al., 2019; Sokol et al., 2019; Rocci et al., 2021). Generally, litter rich in simple forms of C and N compounds (*e.g.,* muramic acid, amino sugars and oligopeptides) tends to increase soil MAOM content, whereas POM is more likely to increase if litter contains more organic matter with large-size molecules (*e.g.,* lignin, chitin and phenols) because their use has a high energy cost for microorganisms to break down (Leifeld and von Lützow 2014; Lavallee et al., 2020; Samson et al., 2020). Soil pH was found important in the formation of MAOM under N addition because soil acidification strongly prohibited microbial biomass increase and SOM decomposition rates (Averill and Waring 2018). Meanwhile, changes in other soil factors induced by N addition may favor microbial growth, *e.g.,* the increase in soil C and N availability. Recent studies have also addressed the connection of soil texture with the formation of POM and MAOM, and higher clay content generally fosters accumulation of MAOM since it can provide more mineral binding sites with microbial and plant-derived compounds than silt and sand (Hicks-Pries et al., 2018; Rasmussen et al., 2018; Chen et al., 2021b; Kleber et al., 2021).

Temperate grassland is facing to be threatened by increasing levels of N deposition largely caused by agricultural fertilization and fossil fuel combustion (Zhang et al., 2016; Niu et al., 2021a,b). In this study, we conducted a field experiment with a set of N addition rates to simulate the N deposition in a temperate grassland of north China. Our first objective was to elucidate the vertical distribution pattern of SOC and total N in different SOM fractions in a temperate grassland by quantifying their contents and stocks in POM vs MAOM at different soil depths (0–10, 30–40 and 70–100 cm). Our second objective was to preliminarily assess the effects of 10-year N addition on SOC and total N contents in soil fractions and to characterize their relationships with soil basic features and soil microbial properties. We also wanted to evaluate the role of extra N input and soil depth in their relative contribution of soil fractions to SOC and total N in bulk soil, which was described as the sum of SOC or total N as POM and MAOM fractions. We hypothesized that: (1) the contents and stocks of SOC and total N in the two fractions all decreased with soil depth, but their proportion might be relatively stable; and (2) the responses of SOC and total N in both POM and MAOM fraction to N addition are different, and that the responses could be further affected by soil depth.

2. Materials and methods

2.1. Study area and experimental design

This study was carried out at the Inner Mongolia Grassland Ecosystem Research Station, which situated in Xilingol, Inner Mongolia of China with coordinates of 116°14′E, 43°13′N and elevation was ~ 1250 m. The site was relatively flat and originally used as a grazing grassland before it was enclosed in 1999. Since then, the grassland was under restoration and there was not any utilization till 2008 when the site was set to N addition treatment. Till our sampling of this study, the site had been treated for more than 10 years consecutively with different levels of N addition. The vegetation is typical steppe with a semiarid climate. Mean annual precipitation is 321.8 mm and mean annual temperature 0.9 °C (1985–2017), with most precipitation and highest temperature occurring in summer time from June to August. Vegetation in this study area is mainly dominated by grasses *Stipa grandis* and *Leymus chinensis* because of their high aboveground biomass contribution (>60 %) in the community. The soil can be categorized as the Haplic Calcisol, with surface soil (0–10 cm) pH 7.4, total carbon and total N 20.1 g kg^{-1} and 2.1 g kg^{-1}, and with concentrations of inorganic N, dissolved organic carbon (DOC) and dissolve organic nitrogen (DON) being 27.8, 50.53 and 18.76 mg kg^{-1}, respectively (Niu et al., 2021a). Total atmospheric N deposition in this region is estimated less than 2 g m^{-2} $year^{-1}$ (Yu et al., 2019).

The field experiment was established in 2008 and N addition treatments have been applied yearly since then. The study site is relatively flat and has been used for livestock grazing until it was fenced in 1999, and has never been utilized by any means since then. The detailed description can be found in detail in Niu et al., (2021a). In brief, the experiment used a randomized block design with 10 blocks and each block contains totally 38 10 m × 10 m plots with 1 m buffer zones as walkways. In this study, 6 out of 10 blocks and 4 N addition rates (50, 10, 2 and 0 g m^{-2} $year^{-1}$) were used to analyze the N addition effects on the C and N stocks in the two soil fractions (POM and MAOM). The four N addition rates were designated respectively as high-N, mid-N, low-N addition and control. All fertilizer treatments in the form of NH_4NO_3 were applied twice a year on the first day of June and November, and no other type of fertilize N, and no other nutrients (*e.g.,* K and Fe) were added in our system.

2.2. Soil sampling and fractionation

In early September (at the end of growing season) 2018, after ten-year consecutive N addition treatments, five samples per soil depth (0–10, 30–40 and 70–100 cm) were taken and composited per field ($n = 6$). To remove visible materials (including big stones, litter and coarse roots), all sampled soil was through a 2-mm screen. For convenience of storage, each composite soil sample was distributed further into some subsamples. One subsample was air-dried, and the other two were stored at 4 °C and −20 °C for later evaluation. Samples applied to determine bulk density (BD) were collected from three points in each sampling plot with cylinders (total volume of 100 cm^{-3}).

To determine the relative contribution of POM vs MAOM in SOC and total N stocks to bulk SOM, we divided each soil sample into POM and MAOM and defined soil fraction with size of 0 ~ 53 μm as MAOM and that with size of 53–2000 μm as POM (Lavallee et al., 2020). Fractionation processes were as follows. Air-dried soil at the weight of ~ 20 g was placed in a 250 ml jar. Each soil sample was sprayed with distilled water to overcome hydrophobicity. To make each soil sample completely dispersed, soil sample was added with 200 ml dilute (0.5 %) sodium hexametaphosphate solution (soil/solution, 1:10) and with ~ 4 g beads. Then the mixed liquor was shaken and beads for 18 h. This approach has no effects on the 0 ~ 53 μm soil fractions. The dispersed soil was sieved using distilled water several times with a 53 μm screen. The fraction left on the screen was regarded as POM while the fraction through the screen was regarded as MAOM (Cotrufo et al., 2019). All the collected soil fractions were stove-dried at 60 °C till constant weight and the amount of each fraction was weighed. The recovery percentages of all soil samples were all higher than 96 % (Table S1).

2.3. Soil physicochemical characteristics and microbial biomass

Soil bulk density in each plot was determined and averaged by treatment with the mass of the two oven-dried soil (105 °C for 48 h) samples. To determination the particle size distribution, each soil sample was oxidized with H_2O_2 first to eliminate SOM. The remaining part was saturated applying $Na_4P_2O_7$. Soil samples were gauged using a Micromeritics Sedigraph (Micromeritics 5100, Norcross, USA) after being shaken and the liquid supernatant removed. The fractions with size of <2 μm, 2 ~ 63 μm and 63 ~ 2000 μm were described as clay, silt and sand, respectively. Chloroform fumigation-extraction method was applied to measure microbial biomass C and N (Vance et al., 1987; Niu et al., 2021a,b). The contents of soil inorganic N, DOC and DON were determined with extracts from 10 g of fresh soil using 50 ml of 0.5 mol/L K_2SO_4, and further measured with a flow injection analyzer (Skalar; Breda, The Netherlands), and a Multi N/C 21005 (Analytik-Jena AG). Microbial PLFAs were determined with extracts from 8 g of freeze-dried soil using a single-phase mixture liquid of chloroform:methanol: phosphate buffer (1:2:0.8 by volume, pH = 7.4), and further analyzed using a gas chromatograph equipped with a flame-ionization detector (Agilent 6850, MIDI V.6.2; Agilent Technologies, Palo Alto, CA, USA). And the details can be found in Niu et al. (2021a,b).

The SOC and total N content in POM and MAOM were measured with a C/N elemental analyzers (Model TruMac, Leco Corp., USA). Prior to elemental analysis, all soil fractions were treated with acids (HCl) to remove the inorganic C. The SOC and N stocks in POM or MAOM were calculated using the following equations (Eqs. (1), (2)):

$$\text{POM - SOC stock}\ \left(\text{kg m}^{-2}\right) = \text{C1} \times \text{M1}/(\text{M}_t) \times \text{BD} \times (1 - \text{RF}) \times t \times 0.01 \qquad (1)$$

$$\text{MAOM - SOC stock}\ \left(\text{kg m}^{-2}\right) = \text{C2} \times \text{M2}/(\text{M}_t) \times \text{BD} \times (1 - \text{RF}) \times \text{t} \times 0.01 \qquad (2)$$

where C1 and C2 are SOC concentrations as POM and MAOM fractions in g kg^{-1}; M1 and M2 are the mass of POM and MAOM fractions in g; M_{total} is the total mass of bulk soil sample; BD is bulk density of each soil layer in g cm^{-3}; RF is the volumetric fraction of rock fragments with the size being larger than 2 mm; t is the thickness of soil layer (10 cm) and the conversion factor is 0.01 for kg m^{-2}. As such, the equations are applied to calculate total N stocks in POM or MAOM when indices were replaced by *N*-related terms. The total SOC and total N content and stock in bulk soil were computed as the sum in both POM and MAOM. We also calculated the proportion SOC and total N in the two fractions using their contents or stocks divided by total content and stock of SOC and total N.

2.4. Statistics

All statistical analyses were implemented in R 3.6.2, 4.1.2, and we firstly tested whether the data meet the requirements for analysis of variance (ANOVA). Then, Additionally, we executed redundancy analysis in "vegan" package twice for each soil layer, where content of SOC or total N in POM and MAOM were regarded as explained variables, and eight soil physicochemical features (including soil pH, total C, total N, DOC, DON, inorganic N, clay content and belowground gross biomass) or soil microbial properties (including content of MBC and MBN, the relative abundance of fungi, bacteria, gram-positive (GP) bacteria, and Gram-negative (GN) bacteria, and the ratios of Fungi: Bacteria (F:B) and GP: GN) were regarded as explanatory variables, respectively. Furthermore, the relative influence of the two type variables in modulating C and N distributions in two fractions were quantified by performing variation partitioning analyses in "vegan". Pearson correlations were applied subsequently to further determine the correlation between contents of SOC and total N in the two soil fractions.

3. Results

3.1. Soil basic features and mass proportion of soil fractions

Soil bulk density, and contents of soil clay, silt, MBC and MBN significantly decreased whereas sand content significantly increased with soil depth (Table 1). N addition had no significant effects on the aforementioned indices in, except for MBC and MBN, which decreased significantly under mid- and high-N addition as compared with the control in the 0–10 cm soil (Table 1). The mass of POM was 11.81 ~ 14.40 g/20 g dry soil while that of MAOM was 5.41 ~ 7.58 g/20 g dry soil (Table S1). Moreover, the POM mass increased while MAOM mass decreased slightly with soil depth (Table S1).

3.2. Contents and stocks of SOC and total N in POM and MAOM

The contents and stocks of SOC and total N in MAOM were significantly higher than those in POM regardless of soil depth and N treatments, and the difference between the two fractions enlarged with increasing soil depth (Figs. 1, 2). The contents and stocks of SOC and total N in POM and MAOM decreased significantly with soil depth, but their contribution to total SOC (Fig. 1) and total N (Fig. 2) in bulk soil were different. The average SOC content in bulk soil was 17.94, 6.26, 2.76 g kg^{-1} in 0–10, 30–40 and 70–100 cm soils, respectively; while the corresponding values of total SOC stock in bulk soil were 2.03, 0.83, 0.38 kg m^{-2} (Fig. 1c and f). The average total N content and stock in bulk soil was 0.41 ~ 2.00 g kg^{-1} and 0.06 ~ 0.23 kg m^{-2} respectively across the soil profile (Fig. 2c and f). The contribution of MAOM for the contents of SOC and total N parallels that for the stocks of SOC and total N (Figs. 1, 2). The contribution of MAOM for SOC and total N stocks (more than 50 % and 55 %, respectively) were generally higher than POM across the soil profile. Moreover, the MAOM contribution increased with increasing soil depth regardless of SOC and total N (Fig. 1c, f; Fig. 2c, f).

Nitrogen addition significantly increased SOC content (+28 %) and stock (+23 %) of POM in the 0–10 cm soil as compared with the control, but largely showed no significant effects in deeper soils (Fig. 1 a, d). Also, N addition did not significantly affect total N contents and stocks in deep soil layers, but increased that in surface soil (0–10 cm) (Fig. 2 a, d). For the MAOM fraction, N addition significantly increased its contents and stocks of SOC and total N in all soil layers, and the increasing effects became more significant with increasing N addition levels (Figs. 1, 2). Overall, N addition increased contents and stocks of bulk SOC and total N in the 0–10 and 30–40 cm soils (all $P < 0.05$), but that was significant only in the high-N addition treatment in the 70–100 cm soil (Figs. 1, 2). The N addition effects on the contribution of SOC and total N in soil fractions depended on N addition levels and soil depth. Specifically, the MAOM contribution of SOC and total N to total SOC and total N in bulk

Table 1
Changes of soil physical features and microbial biomass along soil profile after a decade of N addition.

Depth (cm)	Treatment	BD (g cm^{-3})	Soil particle size distribution (%)			MBC	MBN
			Clay	Silt	Sand		
0–10	Control	1.15(0.02)	5.07(0.03)	45.07(1.37)	49.86(1.35)	179.95(7.60) a	14.03(0.57) a
	Low-N	1.16 (0.02)	5.51(0.23)	47.38(2.16)	47.12(2.38)	162.02 (8.33) a	11.75 (0.59) a
	Mid-N	1.11 (0.02)	5.40(0.27)	45.49(1.77)	49.11(2.02)	134.07 (4.63) b	9.28 (0.37) b
	High-N	1.10 (0.02)	4.97(0.27)	42.88(1.37)	52.15(1.61)	60.00 (3.15) c	6.28 (0.18) c
30–40	Control	1.33 (0.03)	3.27(0.16)	43.74(1.57)	52.99(1.68)	77.11 (3.63)	6.50 (0.31)
	Low-N	1.33 (0.04)	3.37(0.15)	42.74(1.20)	53.89(1.29)	74.65 (3.50)	6.20 (0.23)
	Mid-N	1.31 (0.01)	3.25(0.09)	43.46(1.74)	53.29(1.78)	79.81 (3.17)	6.13 (0.41)
	High-N	1.36 (0.03)	3.58(0.14)	45.34(2.18)	51.08(2.16)	67.59 (2.72)	5.64 (0.34)
70–100	Control	1.34 (0.01)	3.26(0.13)	36.50(1.07)	60.24(1.18)	10.57 (0.49)	3.56 (0.21)
	Low-N	1.38 (0.03)	3.41(0.18)	39.58(1.59)	57.01(1.61)	9.53 (0.53)	3.54 (0.20)
	Mid-N	1.38 (0.01)	3.42(0.13)	37.21(0.62)	59.37(0.72)	9.48 (0.60)	3.62 (0.18)
	High-N	1.39 (0.03)	3.58(0.27)	40.42(1.05)	56.00(0.92)	8.29(0.49)	3.51 (0.21)

Notes, data are presented as mean values and standard error are shown in brackets (n = 6). The lowercase letters indicate significant ($P \leq 0.05$) differences among different treatment in a given soil layer. BD, bulk density; MBC and MBN, microbial biomass carbon and nitrogen.

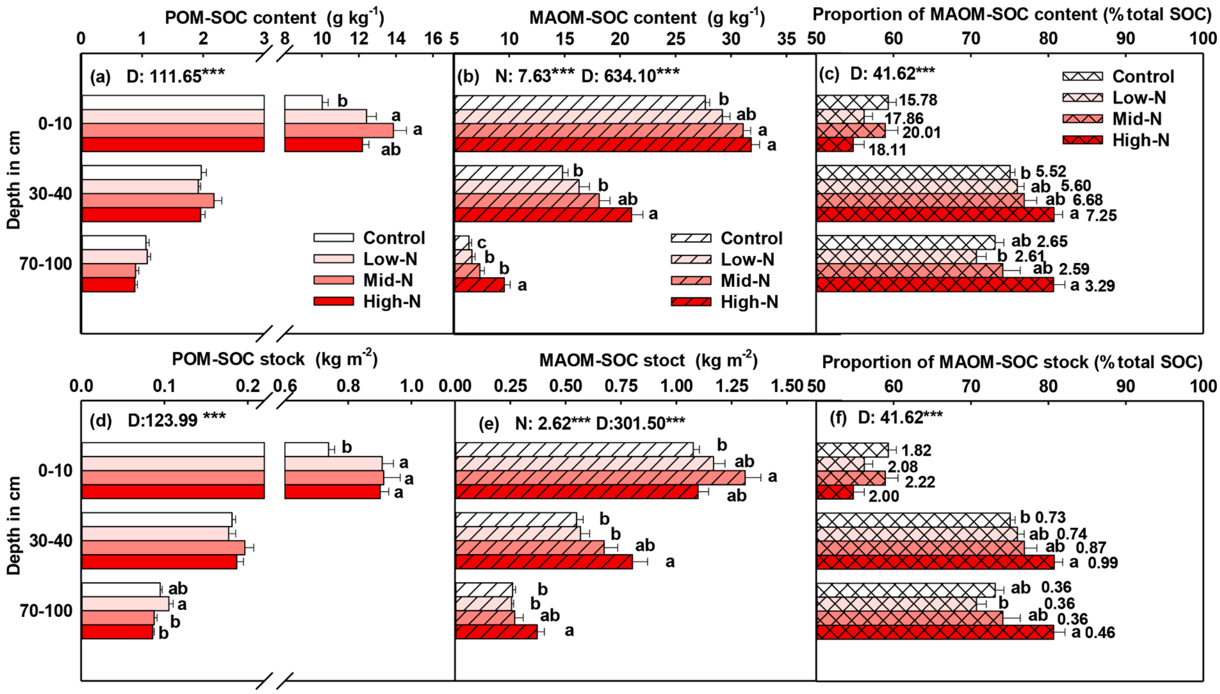

Fig. 1. The SOC contents (a, b) and stocks (d, e) in POM and MAOM fractions and their relative contribution (c, f) to bulk soil (c, f) across the soil profile after a decade's N addition. Notes: The results of two-way ANOVA (F value) of soil depth (D) and N addition treatment (N) for SOC and total N were shown in the top of each plot. Symbols *** indicate extremely significant ($P \leq 0.001$). The number right to the bars in (c) and (f) represent the sum of POM and MAOM. Data in the 70–100 cm soil layer were described as the average of 10 cm soil layer to make them comparable with those in the upper soil layers (i.e., 0–10 cm, and 30–40 cm). See other notes in Table 1.

soil under N addition treatment were generally lower than that of the control in 0–10 cm soil, but the opposite trends were observed in the 30–40 cm and 70–100 cm layers (Figs. 1, 2).

3.3. Factors influencing SOC and total N content in POM and MAOM

The eight soil factors could explain 46.8 %, 69.8 % and 77.3 % of the total variation of SOC distribution between POM and MAOM in 0–10, 30–40 and 70–100 cm layers, respectively (Fig. 3a, c and e), while the eight soil microbial properties explain 22.4 %, 51.1 % and 46.4 % of the total variation, respectively (Fig. 3b, d and f). Variation partitioning analyses indicated soil physicochemical factors exerted stronger controls than microbial properties in the distribution of SOC in the two fractions regardless of soil depth (Fig. S1). In the 0–10 cm soil layer, the contents of soil inorganic N, total C and N significantly affected the SOC distribution in the two soil fractions (Fig. 3a, b). Soil inorganic N content was positively correlated with SOC content of MAOM, while soil total C and N were positively correlated with SOC content of POM and MAOM (Table S2). In the 30–40 cm soil layer, soil pH and contents of soil inorganic N, DON and total N changed the distribution of SOC and total N content in the two soil fractions (all $P \leq 0.05$), while the relative abundance of GP and GN bacteria also significantly influenced the SOC content in POM and MAOM (Fig. 3c, d). Significant positive correlations of soil inorganic N, total N with MAOM SOC content, and soil total C with SOC content of POM and MAOM were also detected (Table S2). The GP relative abundance was positively correlated, whereas GN relative

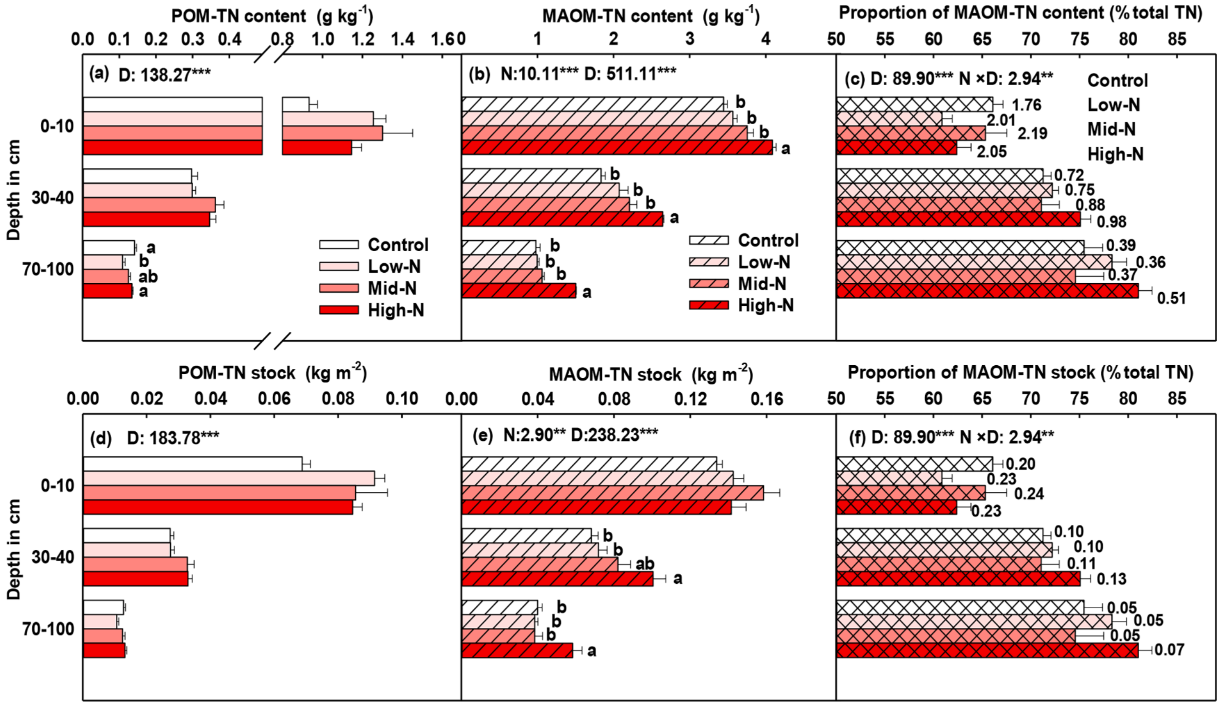

Fig. 2. The total N contents (a, b) and stocks (d, e) in POM and MAOM fractions and their relative contribution (c, f) to bulk soil (c, f) across the soil profile after a decade's N addition. See notes in Fig. 1.

abundance was negatively correlated with SOC content of MAOM ($P \leq 0.05$ in both cases, Tables S2, S3). In the 70–100 cm soil layer, soil pH and the contents of total N, inorganic N significantly changed the distribution of SOC content in POM and MAOM, while the effects for most of the soil microbial properties (including MBC, relative abundance of fungi, bacteria and their ratio) were significant (Fig. 3e, f).

For the distribution of total N content in POM and MAOM, measured soil features explained 31.9 %, 72.1 % and 73.6 % of total variation respectively at three soil layers, while soil microbial properties explained 29.1 %, 57.2 % and 66.0 %, correspondingly (Fig. 3). Variation partitioning analyses also indicated the relative influence of soil physicochemical factors was higher than microbial properties in the distribution of total N in the two fractions regardless of soil depth (Fig. S2). Soil pH and the contents of inorganic N, total N significantly influenced the total N distribution in the 0–10 cm and 70–100 cm soils, while the effects of MBC content and the relative abundance of fungi in 0–10 cm soil were also significant (Fig. 4a, b). Soil pH and the contents of inorganic N, DON and total N of bulk soil were the important moderators in the 30–40 cm soil, while the relative abundance of GP and GN bacteria also influenced the total N distribution ($P \leq 0.05$ for all cases, Fig. 4c, d). The Pearson's correlations of soil features and microbial properties with total N in soil fractions resemble those with SOC in the two soil fractions (Tables S2, S3).

4. Discussion

4.1. *Storage of SOC and total N in POM and MAOM at different soil depths*

By calculating SOC and total N stocks of POM and MAOM fractions across the entire soil profile, we found more SOC and total N were held in MAOM fraction than in POM fraction regardless of N addition and soil depth in the studied grassland. These findings are consistent with the results of a previous study (Cotrufo et al., 2019), which showed that MAOM was the dominant SOM pool in the topsoil (0–20 cm) in European grasslands although no data available in deeper soils. Moreover, both the contents and stocks of SOC and total N in MAOM and POM fractions all decreased with soil depth, but the proportion of SOC and total N as MAOM to these in bulk soil increased. The decrease in SOC and total N with soil depth are as expected because of reduction in the inputs of plant materials (including litter, plant roots and root exudates) and contents of microbial biomass, DOC and DON (Rumpel and Kögel-Knabner et al., 2011; Muhammad et al., 2017; Hicks-Prices et al., 2018). Our results emphasize the importance of deeper soil for the storage of SOM in grassland ecosystems, especially as MAOM fraction. This is because a high quota of SOC is present as MAOM in deep soil, and the proportion of stable SOM in the first 1 m of the soils is higher than we thought before (Cotrufo et al., 2015; Angst et al., 2018; Balesdent et al., 2018).

There are several reasons currently available to explain why more SOC and total N are reserved as MAOM rather than POM and why the MAOM portion increased with soil depth in our studied grassland. Firstly, POM predominantly contains relatively recalcitrant plant-origin or fungal-derived matter (*e.g.,* lignin, chitin) whereas MAOM is largely composed of microbial products, such as amino sugars, microbial polysaccharides and muramic acid (Cotrufo et al., 2015; Lavallee et al., 2020). In our studied grassland, litter quality is relatively high because the plant community largely consists of grasses, which contains many non-structural carbon compounds, and thus most plant residues are decomposed by soil microbes within a few years (Zhang et al., 2016; Hou et al., 2021). Consequently, only a small amount of recalcitrant matter accumulates on the soil surface and further move to deep soil by leaching. Secondly, the modulators of nutrient cycling in this grassland are dominated by bacteria, which speed up nutrient cycling and promote MAOM formation (Crowther et al., 2019). Our previous study showed that bacteria relative abundance was more than 70 % even at 70–100 cm soil layer despite its decrease with soil depth (Niu et al., 2021a). The dominance of bacteria could work in concert with the high litter quality as bacteria can predominantly participate in the decomposition of relatively more degradable matter than fungi (Liang et al., 2017; Crowther et al., 2019). Importantly, topsoil MAOM formation reflects aboveground plant inputs and litter decomposition whereas subsoil MAOM indicates the contributions of dissolved organic matter, plant roots, root exudates and bioturbation (Rumpel and Kögel-Knabner,

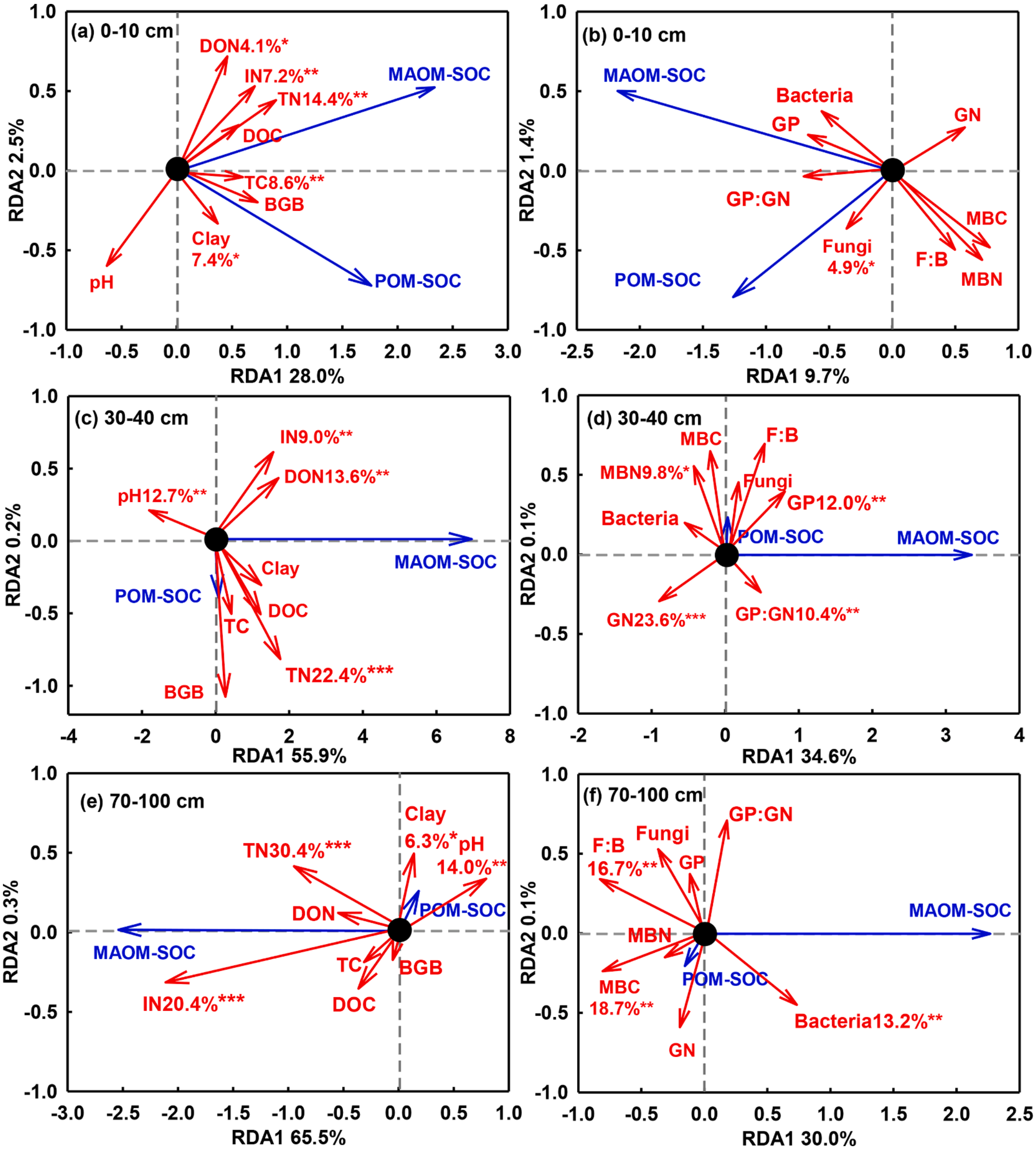

Fig. 3. Relationships of SOC content with soil physicochemical features (a, c, e) and microbial properties (b, d, f) across the soil profile after a decade's N addition. Notes, DON, dissolved organic N; DOC, dissolved organic C; IN, inorganic N; BGB, belowground gross biomass; GP, Gram-positive bacteria; GN, gram-negative bacteria; MBC, microbial biomass C; MBN, microbial biomass N; F: B, fungi: bacteria ratio. Blue arrows represent SOC content in POM and MAOM; red arrows represent soil physicochemical variables or the relative abundance of microbial PLFAs. While *, ** and *** indicate significant effects of these variables at $P \leq 0.1$, 0.05, 0.01, respectively, the values show the relative importance of each variable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2011; Neurath et al., 2021). Thirdly, the higher proportion of SOC and total N as MAOM in our studied grassland with its semiarid climate are more closely associated with soil texture and minerals. Recent studies have showed the roles of soil clay and mineral elements (i.e., Ca, Fe and Al) in SOM pools and suggested that mineral protection may control the formation of fine-sized SOM fractions (Fang et al., 2018; Chen et al., 2021b). Moreover, high contents of mineral elements generally favor MAOM formation. In this study, although we did not determine the effects of Al/Fe-(hydr) oxides and Ca on SOM formation directly, we also found that the total contents of Al, Ca and Fe in MAOM were significantly higher than those in POM, especially in deeper soil layers (Fig. S3). This may indicate that the effects of mineral protection in subsoil is equal to or even exceeds that in topsoil. This indication is also supported by another recent study in that mineral protection related to Fe-Al oxides and cations in SOM presence became more important in subsoil (Chen et al., 2021).

4.2. *Effects of N addition on storage of SOC and total N in POM and MAOM*

Our results demonstrate that the effects of extra N input on the contents and stocks of SOC and total N were remarkably different between the two soil fractions, and the effects were also modified by soil depth. The increase in the amount of SOC respectively in POM, MAOM and bulk soil in the surface 0–10 cm layer after N addition corroborated results of a meta-analysis on the SOM fractions (Rocci et al., 2021), which showed that N fertilization increased SOC in all SOM fractions in the topsoil. In this study, we found that the increasing effects of N addition on SOC and total N in POM were stronger than that in MAOM for the surface soil layer, indicating that the MAOM proportion of SOC and total N decreased because of N addition. As was discussed above, changes in plant biomass and species composition of communities under N addition may help to illustrate the increase of SOC and total N of both

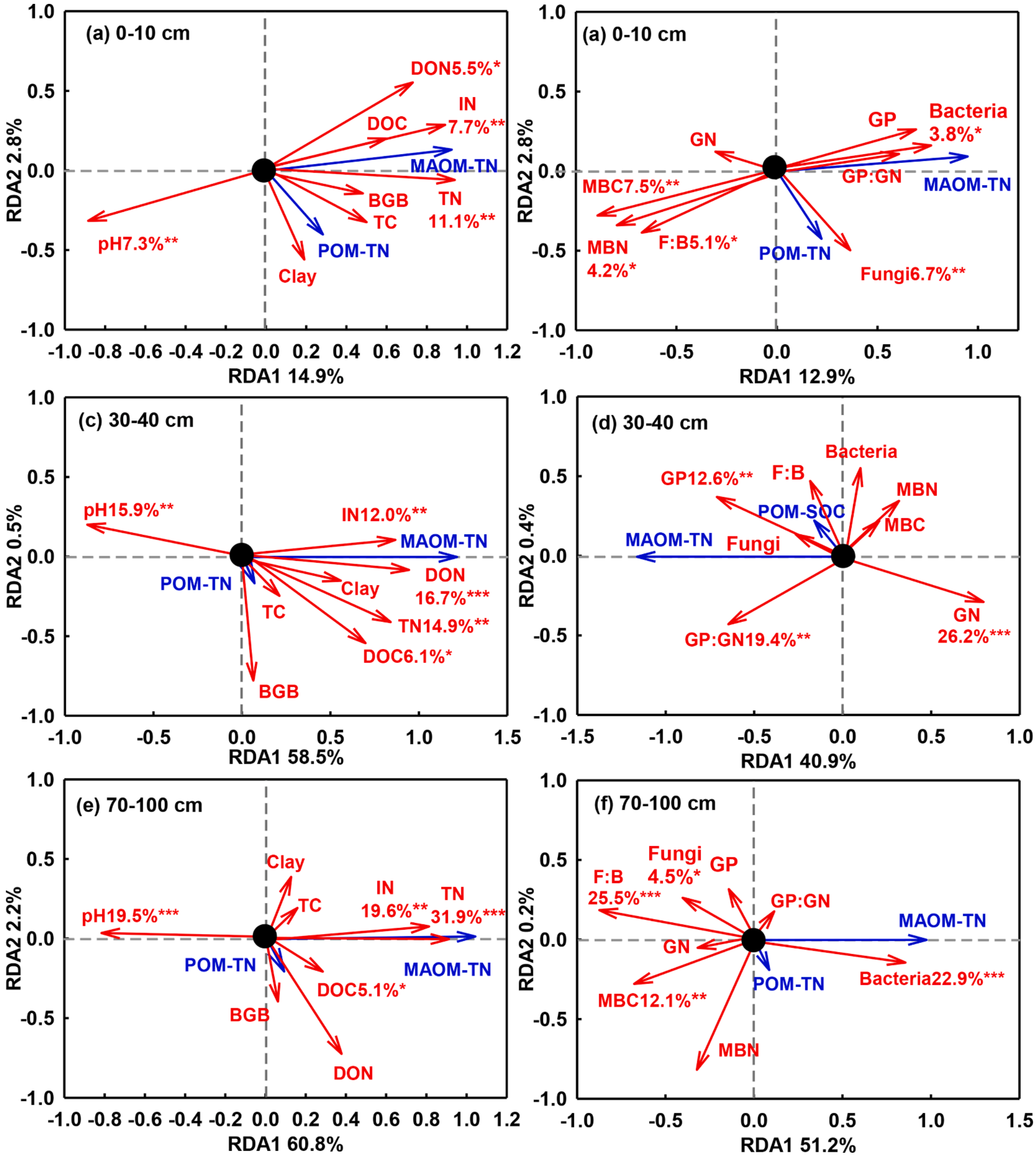

Fig. 4. Relationships of total N content with soil physicochemical features and microbial properties across the soil profile after a decade's N addition. See notes in Fig. 3.

soil fractions in the topsoil. On the one hand, previous studies in the same experimental site indicated that N inputs increased plant biomass and therefore litter mass, although the degree of influence depended on the amount of precipitation (Zhang et al., 2015; Ren et al., 2021). Higher accumulation of plant residues after N inputs could provide more organic matter for the formation of POM and MAOM. On the other hand, N addition significantly altered plant community species composition and further influenced the overall litter quality by enhancing the dominance of *Agropyron cristatum* and *Leymus chinensis* but reducing the dominance of *Stipa grandis* and *Achnatherum sibiricum*as which have higher concentrations of lignin, cellulose and hemicellulose than *A. cristatum* and *L. chinensis* (Zhang et al., 2015; Hou et al., 2021). Thus, the increasing response of SOC and total N in POM of topsoil to N addition is likely the result of an increased input of non-structural plant residues to topsoil (Xia and Wan, 2008; Hou et al., 2021). Additionally, soil microbes may prefer to use the non-structural plant residues and suppress degradation of recalcitrant matter under N addition, thus simultaneously fostering the accumulation of topsoil POM and MAOM (Rinkes et al., 2016; Hou et al., 2021).

In the deeper soil layers (30–40 and 70–100 cm), our results showed that N addition increased MAOM SOC and total N and their proportion in bulk soil, although the increase was significant only under high-N addition. Unlike in topsoil where the organic matter is largely from aboveground plant litter, the sources of organic matter in subsoils generally include dissolved organic matter, plant roots and root exudates (Rumpel and Kögel-Knabner, 2011; Harper and Tibbett, 2013). However, the grasses in our study system have relatively shallower (0–20 cm) root profiles, and the root biomass of 0–20 cm soil could explain more than 90 % of the total belowground root biomass within the 1 m soil profile. Our previous studies showed that extra N input significantly enhanced the contents of DOC, DON and inorganic N even in the 70–100 cm soil layer and promoted accumulation of base cations (*e.g.,* Ca, Mg) in deep soils (Niu et al., 2021a,b), indicating that leaching was an important soil physical process. We thus conclude that the increase of dissolved organic matter can be the primary source of SOC and TN for MAOM under N addition, which were supported by our results of redundancy analysis (Fig. 4). The interpretation is seemingly unrealistic because the annual precipitation is generally less than 350 mm in the study area. We analyzed the precipitation data during the period from 2008 to 2017 and found that most of the yearly precipitation occurred in a few days with the daily precipitation being more than 30 mm (data not shown). This suggests remarkable leaching could occasionally happen during these heavy episodes of precipitation. A previous study across observing meteorological stations worldwide has also shown that the wettest 12 days can take up half of the annual precipitation each year (Pendergrass and Knutti, 2018).

Redundancy analysis and Pearson correlation analysis also indicated that DOC and DON were the important moderators especially in subsoils, and that the total N, DOC, DON and inorganic N were positively correlated with SOC and total N in MAOM (Figs. 3, 4; Tables S2–S3). These results further hint to the importance of leachate in the accumulation processes of SOC and total N in subsoils. Unexpectedly, we did not find that soil pH and microbial properties significantly affected SOC distribution between POM and MAOM in the 0–10 cm, but significantly did so in the 30–40 and 70–100 cm soils (Fig. 3, Fig. S1-2). The insignificant effects of soil pH and microbial properties in 0–10 cm soil may be due to the fact that the topsoil receives amounts of non-structural plant matter, and the effects of chronic plant inputs since the N addition effects may override the effects of soil pH and microbial properties (Fig. S1-S2). Indeed, plant carbon input has been shown to control topsoil carbon destabilization (Chen et al., 2021b; Witzgall et al., 2021). In addition to soil physicochemical features, soil microbial properties also influenced the distribution of TN in fractions although their relative influence was generally low (Figs. 3, 4). The exact reasons that caused these aforementioned results are elusive yet, but it is highly notable that changes in soil microbial composition could alter the distribution of SOC and TN in fractions as a result from different microbial groups (Aumtong et al., 2011; Liang et al., 2017; Bastida et al., 2021; Boeddinghaus et al., 2021). Our results indicate that the relative abundance of bacteria vs fungi, GP vs GN and the ratio of the two counterparts were important moderators (Figs. 3, 4) since bacteria and/or GN would preferably use the non-structural matter more than fungi and GP (Niu et al., 2021a). However, the correlation of these microbial parameters with SOC and total N in POM or MAOM had no consistent pattern. A possible explanation is that we cannot accurately determine the change in soil microbial composition solely based on PLFAs. Taken together, N addition influenced the relative distribution of SOC and total N by altering the amount and quality of plant residues in soil fractions of topsoil, but by affecting the amount of dissolved organic matter in subsoils.

In this study, we emphasized the importance of MAOM in SOC stabilization due to its high SOC and total N contents, even though the mass of MAOM fraction is less than 40 % across the soil profile in the studied grassland. However, there are limitations about our results that need more studies to verify and validate. Specifically, changes of topsoil and subsoil properties with long-term N inputs in our study were insufficient to fully understand the mechanism of POM and MAOM formation. Based on results of recent studies, two relevant issues still require more attention for future studies (Averill and Waring 2018; Robertson et al., 2019; Kleber et al., 2021; García-Palacios and Chen 2022). First, more advanced techniques (*e.g*, next-generation sequencing) should be combined with PLFA analysis to explore how shifts of soil microbial communities and enzyme activities influence the formation processes of POM and MAOM with critical plant inputs. Second, mineral protection by Fe-Al oxides, cations by Ca, and the sorption–desorption processes between POM and MAOM at different soil layers remain largely unclear.

5. Conclusions

To summarize, this study shows that the MAOM fraction is the dominant pool of SOC and TN regardless of soil depth and N addition in this grassland, while deep soil has a higher proportion of SOC and TN as MAOM than topsoil. These finding suggests that the implementation of SOM sequestration, especially in the restoration of degraded typical steppe, requires full consideration of depth-specific soil and the relative distribution of SOC and total N in the two soil fractions (POM and MAOM). After 10-year of continuous N addition, both the contents and stocks of SOC and TN in the two soil fractions increased in topsoil, while increased only in MAOM in deeper soil layers (*e.g.,* 30 cm or deeper). Soil physicochemical features are the predominant driver over soil microbial properties for the distribution of SOC and total N in SOM fractions. These findings imply that an increase in N deposition may make more SOC stabilized as MAOM fraction of grassland soils. Thus, its saturation level in *N*-limited grassland ecosystems should be targeted in further studies and considered in predictions of SOM dynamics.

Data availability statement

The data used for this article can be found in https://doi.org/10.6084/m9.figshare.19070282, and the DOI becomes active when article is published.

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.ecolind.2022.109471.

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