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PRIMARY RESEARCH ARTICLE

Long-term (64 years) annual burning lessened soil organic carbon and nitrogen content in a humid subtropical grassland

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ABSTRACT

Burning has commonly been used to increase forage production and nutrients cycling in grasslands. However, its long-term effects on soil organic carbon (SOC) and nitrogen (N) pools within the aggregates and the relation between aggregates-associated SOC and soil CO₂ emissions need further appraisal. This study evaluated the effects of 64 years of annual burning on SOC and N dynamics compared to annual mowing and undisturbed treatments in a grassland experiment established in 1950. Soils were sampled from four depths representing the upper 30 cm layer and fractionated into macroaggregates, microaggregates and silt + clay fractions. The macroaggregates were further fractionated into three occluded fractions. The SOC in the bulk soil and aggregates were correlated to soil CO₂ effluxes measured under field conditions. Compared to the undisturbed treatment, annual burning decreased aggregates stability, SOC and N in the upper 30 cm layer by 8%, 5% and 12%, respectively. Grassland mowing induced greater aggregates stability than burning only in the upper 5 cm. Burning also decreased SOC in the large macroaggregates (e.g., 0–5 cm) compared to mowing and the undisturbed grasslands but proportionally increased the microaggregates and their associated SOC. Soil N associated with aggregates decreased largely following grassland burning, for example, by 8.8-fold in the microaggregates within the large macroaggregates at 20–30 cm compared to the undisturbed grassland. Burning also increased soil CO₂ emissions by 33 and 16% compared to undisturbed and mowing, respectively. The combustion of fresh C and soil organic matter by fire is likely responsible for the low soil aggregation, high SOC and N losses under burned grassland. These results suggested a direct link between grass burning and SOC losses, a key component for escalating climate change severity. Therefore, less frequent burning or a rotation of burning and mowing should be investigated for sustainable grasslands management.

KEYWORDS

annual mowing, climate change, grassland sustainability, soil aggregates, soil respiration, South Africa

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1 | INTRODUCTION

Grasslands occupy ~40% of the global land surface, represent up to 70% of the agricultural area, and store about 20% of the total terrestrial carbon (C) (Blair et al., 2014; Conant, 2010; Ramankutty et al., 2008), and are thus crucial for climate change mitigation. However, the aggravating climate change risk associated with inappropriate management practices significantly influences the mitigation potential of grasslands (Chang et al., 2021; Nandintsetseg et al., 2021). Therefore, there is an urgent need to adopt strategic grassland management practices to offset greenhouse gas emissions by increasing soil organic matter (SOM), a key component for grassland sustainability. The interaction of management practices, soil properties and climatic conditions can determine the long-term fate of SOM. Several improved grassland management practices were found to increase C inputs and it is stabilized in soils (Chaplot et al., 2016; Chen et al., 2015; Whitehead, 2020). The C is mainly stabilized in the soil aggregates, with the level of stabilization depending on the soil aggregation controlled, mainly by the management practices (Egan et al., 2018; Six et al., 2002). Management practices such as grassland burning and mowing can strongly affect soil aggregation and soil CO₂ emissions, thus alter the soil C stocks of managed grasslands (Abdalla et al., 2016; Shimoda & Takahashi, 2009; Soong & Cotrufo, 2015).

Burning has long been used as traditional management to enhance grassland productivity and prevent bush encroachments into pastures worldwide (Hall & Scurlock, 1991; Montané et al., 2007; Trollope, 1980). Frequent burning directly reduces fresh C inputs to the soils by aboveground biomass and surface litter combustion (Pellegrini et al., 2020). A small proportion (about 4%) of the biomass returned to the soil as a pyrogenic organic matter (also called black carbon and charcoal) alters C and nitrogen (N) cycles (Forbes et al., 2006; Knicker et al., 2012; Soong & Cotrufo, 2015). Another indirect effect is induced by fire severity on the soil properties, that is, soil aggregates stability, C and N pools (Araya et al., 2016, 2017; Grogan et al., 2000; Harris et al., 2008). In a laboratory incubation, Araya et al. (2016), Araya et al. (2017) identified a fire temperature threshold of 350–450°C to cause a significant decline in aggregate stability associated closely with a loss of C and N from macro- and microaggregate fractions in the Sierra Nevada, Spain. High flaming temperature combusts the SOM that binds soil aggregates, leading to aggregates collapse (DeBano, 1981), thus exposes the initial protected SOM to microbial mineralization (Six et al., 2002; Zhao et al., 2012) and soil erosion (Fox et al., 2007; O'Dea & Guertin, 2003), resulting in SOC and N losses. However, most studies on the effect of fire on soil aggregates and the associated C and N under field conditions have been limited to the top 10 cm depths (Armas-Herrera et al., 2016; Fynn et al., 2003; Shimoda & Takahashi, 2009).

Mowing is another commonly used grassland management practice, also affects soil aggregation, and the C and N turnover within the aggregates either directly by increasing return C to the soils or indirectly by stimulating species richness, grass productivity, roots biomass and exudates (Cong et al., 2014; Fynn et al., 2005; Li et al., 2017; Socher et al., 2012). The effect of mowing on SOM

accumulation depends on its frequency, for example, mowing every 1 or 2 years significantly enhanced the SOM accumulation and stabilization compared to twice a year in a semiarid grassland in China (Li et al., 2017). However, Nüsse et al. (2018) found that five cuts per year increased the C and N stocks in the soil and the proportion of macroaggregates fractions at the 0–10 cm soil depths in Germany, an effect explained by the high roots concentration and exudates related to *Lolium perenne* under mowing. Several other studies (e.g., Benning & Seastedt, 1997; Kitchen et al., 2009; Shimoda & Takahashi, 2009) also reported greater roots biomass and fine roots distribution in upper soil depths (i.e., 0–5, 0–10 cm) under mowed compared to burned grasslands. These studies only looked at the effect of mowing on soil aggregates and their associated SOC and N in the upper 5 cm depth, while burning grasslands or leaving them undisturbed could promote C in the deeper soil layers.

Despite the clear evidence that burning and mowing alter soil aggregation globally, few studies have simultaneously addressed their effect on the fate of C and N content in soils. However, their impact on soil C and N within the aggregates particularly that occluded within the macroaggregates after 64 years of a continuous annual application is yet to be investigated. The lack of such studies not only in the Southern African countries but rather the whole sub-Saharan African region makes the current research important to both local and international scientific communities. Therefore, the present study investigated the proportion of aggregate fractions and the SOC and N content associated with these fractions in a long-term grassland experiment established in 1950 at the Ukulinga research farm in South Africa. The obtained SOC data were correlated to soil CO₂ emissions measured at the same site by Abdalla et al. (2016) to address the potential effect of SOC in different aggregate fractions on soil CO₂ emissions. The study hypothesized that first, long-term annual burning would decrease aggregate stability, and C and N pools in the soils compared to annual mowing and undisturbed grassland because the combustion of aboveground biomass and litter reduces fresh C input to soils. Second, long-term annual burning would reduce the proportion of macroaggregates and their associated SOC and N content but increase the proportion of microaggregates in the bulk soil and the occluded aggregates compared to mowing and undisturbed treatments. Mainly because of the disaggregation caused by the combustion of SOM, a binding agent for soil aggregates due to fire effects. Third, an increase in soil CO₂ emissions is expected to be related to the reduction of aggregate stability, that is, more macroaggregates due to the reduced physical protection of SOC.

2 | MATERIALS AND METHODS

2.1 | Study site

The experimental site is located at Ukulinga training and research farm of the University of KwaZulu-Natal, Pietermaritzburg campus, South Africa (24° 24'E, 30° 24'S; Figure 1). The experiment is suited on top of a small sloping plateau with an altitude ranging from 847 to

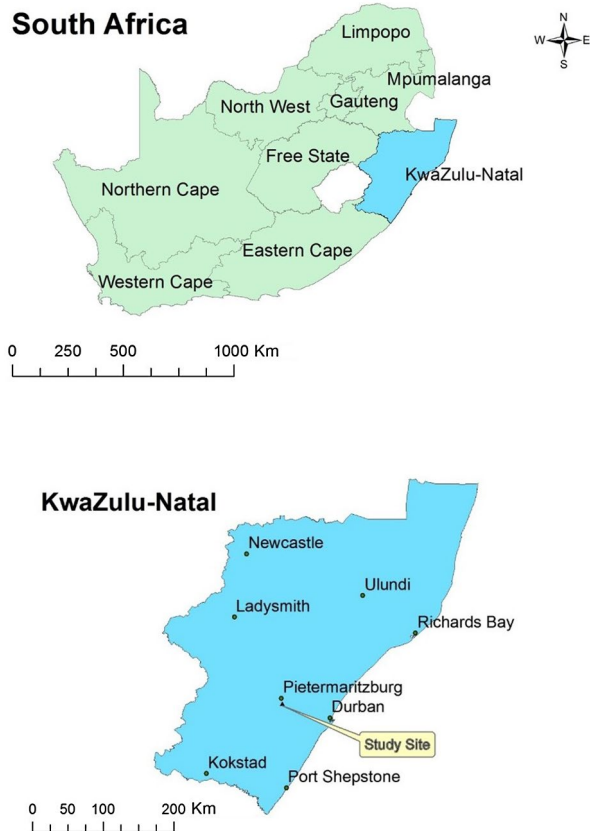


FIGURE 1 The study site location; Pietermaritzburg, KwaZulu-Natal province, South Africa

838 m (Fynn et al., 2004). The soils are derived from colluvium shale with dolerite intrusions and are classified as *Plinthic Acrisols* (IUSS-WRB, 2014). Soil depth is shallow, ranging from 5 cm in the upslope to a maximum of 60 cm depth in the downslope (Abdalla et al., 2016). The soil in the upper 20 cm is acidic ($\text{pH}_{\text{KCl}} = 5.5$), with a silty clay loam texture (37% clay, 43% silt and 20% sand), and an average of SOC and N content of 27.8 and 1.9 g kg soil⁻¹, respectively (Abdalla et al., 2016).

The area has a humid subtropical climate with a mean annual precipitation of 694 mm, about 73% of it occurs between October and March (Fynn et al., 2005; Mengistu et al., 2016). The mean annual temperature is 18.4°C, with monthly minimum and maximum temperatures of 1.5°C in July and 30°C in January, respectively (Mengistu et al., 2016). The native vegetation type is described as dense close grass (0.5 and 0.75 m tall) dominated by southern tall grassveld (Fynn et al., 2004). In the absence of burning, the vegetation had some scattered trees, that is, *Acacia sieberiana*. The native grass species are *Themeda triandra* and *Tristachya leucothrix*, which use the C4 photosynthetic path (Fynn et al., 2004, 2005).

2.2 | Experimental design and treatments

The experimental site was established in 1950 in a virgin Tall Grassveld to investigate the effect of burning and mowing on

grassland diversity and productivity (Tainton et al., 1978). The experiment consists of different burning frequencies, that is, annual and biennial burning at different times of the year (e.g., winter and spring) and annual mowing (e.g., winter and spring), replicated three times and laid out in a complete randomized block design, with 18.3 m * 13.7 m plots separated by 4 m sidewalks (Abdalla et al., 2016; Fynn et al., 2003; Tainton et al., 1978). Hence, annual burning and mowing of grasslands at the beginning of the spring season (i.e., early August) are common grasslands management practices across the South African grasslands (Joubert et al., 2014; Morris et al., 2021; Oluwole et al., 2008). The current study focused on annual burning and mowing in early spring, that is, 1st week of August, since 1950 compared to an undisturbed control that was neither burned nor mowed. The same treatments were utilized previously by Abdalla et al. (2016) to investigate the seasonal dynamics of soil CO₂ emissions. The annual burning plots were dominated by sparse kangaroo grass (*Themeda triandra*) at the time of the study. The fire was usually applied on the grasses by initiating a high intensity back burn using point source ignition on the downwind side of the plots forming a line that spread slowly with a flame height of 1–1.5 m in the wind direction. Burning eliminates all the aboveground biomass and litter, leaving only partially burned grass crowns, white ashes and charred materials, hence classified as severe prescribed burning (Keeley, 2009).

In the annual mowing plots, the grass was cut mechanically, and the clipped grass material was removed from the plots, leaving a 5–8 cm stubble layer with a partially disturbed litter layer. In the undisturbed plots, there has been neither burning nor mowing since 1950. At the time of this study, the undisturbed plots were dominated by spread trees, mainly *Acacia sieberiana* species, which cover about 50% of the plot sizes. The experimental site is surrounded by a 1.5 m chain-linked wire fence to prevent the field from animal grazing.

2.3 | Soil samples collection and preparation

Soil samples for bulk density, aggregates fractionation and chemical analysis were collected in May 2014 from three randomly dug pits (0.5 * 0.5 * 0.3 m) in each plot from four soil depths; 0–5, 5–10, 10–20 and 20–30 cm. Bulk density soil samples were collected from each soil depth using a metallic cylinder (7.5 cm diameter and 5 cm height), which was oven-dried at 105°C, and the ratio of dry soil mass to the volume of the cylinder was used to compute soil bulk density (Grossman & Reinsch, 2002). Another undisturbed soil samples of around 500 g were collected in a cardboard box from each depth using a knife and spade and reserved for the analysis and aggregate fractions. Subsamples for aggregate fractionation were sieved through 8 mm sieve by gently breaking the soil clods before air-drying for 72 h. For C and N analyses, representative subsamples of 20 g of the whole soil sample were taken and stored at room temperature. The rest of the samples were returned to the same cardboard box and reserved for aggregate separations. Due to depth variations between the plots, the 10–20 cm depths were

sampled in two blocks (2 and 3) and 20–30 cm soil depth was only available in the downslope area (block 3).

2.4 | Soil fractionation

2.4.1 | Part-I

Four water-stable aggregate fractions, large macroaggregates (>2 mm), small macroaggregates (2–0.25 mm), microaggregates (0.25–0.053 mm) and silt + clay (>0.053 mm), were separated by the wet sieving method as described by Elliott (1986) (Figure 2). A subsample of 80 g was soaked in 100 ml deionized water for 5 min in a glass beaker and poured on top of 2 mm sieve positioned inside a dishpan half-covered with deionized water. The wet sieving process includes moving the sieve up-down (50 times) in the water with a slight angle, ensuring that the water and small particles go through the mesh. The materials that remained on the top of the 2 mm sieve, representing the large macroaggregates, were collected by backwashing the sieve into a pre-weighted aluminum-drying pan. The soil material and water passed through the 2 mm sieve, poured into a 0.25 mm sieve, and the above sieving procedure was repeated two times to separate the small macroaggregates and microaggregates fractions. Eventually, the material passed through the 0.053 mm sieve represents the silt + clay fraction. The four separated aggregates fractions plus the water were oven-dried at 60°C until a constant weight was achieved. Hereafter, the dry weight of each aggregate fraction was used to calculate the proportion of these aggregates in the bulk soils, expressed as a percentage of the dry soil. The mean weight diameter (a proxy of soil water aggregates stability) for each treatment and soil depth was calculated using the following equation (Kemper & Rosenau, 1986):

$$\text{MWD} = \sum_{i=0}^n x_i w_i \quad (1)$$

where MWD is the mean weight diameter, x_i is the mean diameter for each fraction size, w_i is the proportional weight of the fraction from the total dry weight of the bulk soil and n is the number of aggregate classes separated.

2.4.2 | Part-II

The large and small macroaggregates fractions were further separated into three occluded fractions: coarse particulate organic matter (cPOM, >0.25 mm), microaggregates (mM, 0.25–0.053 mm) and silt + clay (s + cM, <0.053 mm) within the macroaggregates as outlined by Six et al. (2000). In brief, a 5 g subsample of the large macroaggregates was soaked overnight in 50 ml deionized water. The suspension was then transferred to a top of a 0.25 mm sieve screen along with 50 metal beads (4 mm diameter). The sieve was mechanically shaken for 5 min at 250 rpm with continuous water flow to immediately wash the microaggregates and the silt + clay into a 0.025 mm sieve. The material that remains on the top of the 0.25 mm sieve represents the cPOM (Six et al., 2000). Thereafter, the 0.053 mm sieve was moved up and down in the same way as in Part-I to ensure all the silt + clay passed through the 0.053 mm sieve while the material remained on the top of the 0.053 mm, sieve represents the microaggregates. The procedure was conducted separately for the large and small macroaggregates, yielding three occluded fractions from each. All the obtained fractions (Part-I and II) were oven-dried at 60°C until constant weight achieved and stored for C and N analyses.

2.5 | C and N analyses and stocks calculations

Subsamples from bulk soils and aggregate fractions were air-dried, sieved through 2 mm, grounded and analyzed for total C and N

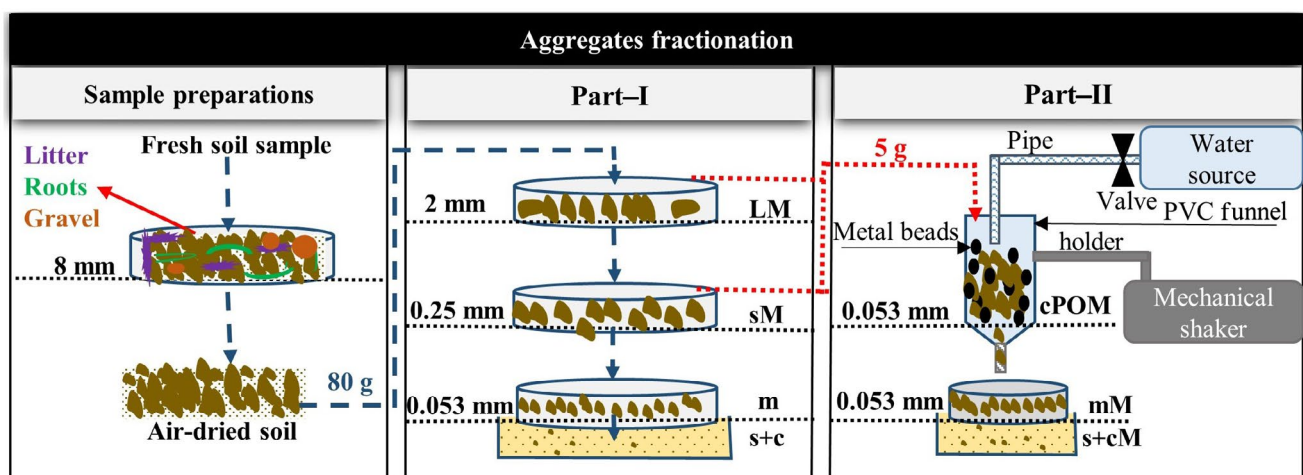


FIGURE 2 Aggregates fractionation diagram. Part-I; fractionating bulk soil into four aggregate sizes (large macroaggregates [LM; >2 mm], small macroaggregates [sM; 2–0.25 mm], microaggregates [m; 0.25–0.053 mm] and silt + clay [s + c; <0.053 mm]). Part II; fractionating the macroaggregates (Lm and sM) from part I into three fractions (coarse sand and particulate organic matter [cPOM; >0.25 mm], microaggregates [mM; 0.25–0.053 mm] and silt + clay [s + cM; <0.053 mm])

using Leco CNS-2000 Dumas dry matter combustion analyzer (LECO Corp.) as described by Kowalenko (2001). In brief, the Leco CNS-2000 used the dry combustion of the solid samples in a horizontal oven that can be heated up to 1450°C. As recommended by Kowalenko (2001), 0.2 g soil was weighted in the automatic sampler and mixed with 1 g catalyst, that is, Leco compact material. The oven temperature was adjusted to 1350°C based on the manufacturer's recommendation. Replicated samples from each treatment and soil depth were measured, and the C and N were determined using an infrared detector cell. Hence, no reaction occurred when drops of HCl (1 M) were added to the soil samples, the C measured were considered as SOC. The SOC and N stocks (SOCs and Ns) were calculated using the SOC content, and soil bulk density in each soil depth following the equation by Batjes (1996):

$$\text{SOCs} = \text{SOCc} \times \rho b \times T \left(1 - \frac{\text{PF}}{100}\right) b \quad (2)$$

where SOCs is soil organic carbon stocks (kg C m^{-2}), SOCc is soil organic carbon content in the ≤ 2 mm soil material (g C kg soil^{-1}), ρb is the bulk density of the soil (kg m^{-3}), T is the thickness of the soil layer (m), PF is the proportion (%) of fragments of >2 mm and b is a constant equal to 0.001.

The nitrogen stocks (Ns) were calculated using the same equation by replacing SOCs and SOCc with soil Ns and N content (Nc), respectively.

2.6 | Soil CO₂ measurements

Soil CO₂ emissions were measured from 10 random points per plot once a month from March 2013 to March 2015. The measurements were done using LI-COR 6400 gas exchange system (LI-COR) fitted with the LI-COR 6400-09 soil respiration chamber. The seasonal variations in the soil CO₂ emission between the treatments were published by Abdalla et al. (2016). In the current study, the daily average of the soil CO₂ emissions measured in 2014 from the treatments was correlated to SOC in the bulk soil and aggregates fractions. This was done using linear correlation coefficients based on the computed average value of the plots ($n = 9$) for each parameter, that is, soil CO₂, total SOC and SOC associated with the different aggregates fractions.

2.7 | Statistical analysis

The experimental data were found to be normally distributed ($p > .05$) using the Shapiro-Wilk normality test. Variations in soil properties caused by grassland management as main effects were tested with one-way analysis of variance (one-way ANOVA) in the 0 LI-5, 5 LI-10, 10 LI-20 cm soil depths ($N = 9$). Similarly, aggregate size fractions in the whole soil, large macroaggregates, small macroaggregates and their associated C and N content in each soil

depth were tested. Due to the lack of replications at 20–30 cm soil depth, the data were analyzed using pseudoreplicates ($N = 3$) with the treatments representing the main effect on soil properties, aggregates sizes, and C and N associated with the aggregates fractions and the pseudoreplicates representing the random effects. All the means were compared using Tukey corrections for multiple comparisons, with a significant threshold at $p \leq .05$, unless specified otherwise, and the variations were documents using the standard error of the means. Pearson correlation coefficients (r) to determine the relationship between soil CO₂ emissions and SOC in the bulk soils, aggregates fractions, and the occluded aggregates were conducted. All the statistical analysis was carried out using the GENSTAT 14th Edition (VSN International), and the figures were produced using SigmaPlot version 12 (Systat Software, Inc.).

3 | RESULTS

3.1 | Soil properties

After 64 years of continuous annual burning and mowing of grasslands, aggregates stability (expressed as a mean weight diameter), bulk density, SOCc and Nc varied greatly compared to the undisturbed treatment (Figure 3). Long-term grassland burning decreased mean weight diameter by an average of 8% in the studied soil depths compared to the undisturbed (Figure 3a). Compared to burning, annual mowing induced a greater mean weight diameter in the upper 5 cm layer, with no significant differences observed at 5–10 and 10–20 cm soil depths. However, at the 20–30 cm depth, burning was associated with a 35% greater mean weight diameter than mowing treatment. Soil bulk density at the 0–5, 5–10 cm depths increased in the following order: undisturbed < burning < mowing (Figure 3b). At the 10–20 cm depth, the undisturbed treatment increased soil bulk density by 7.5% compared to the average of burning and mowing but decreased by 11% compared to burning at 20–30 cm depth.

The SOCc and SOCs decreased with soil depth in all treatments, with always greater values in the undisturbed than burning and mowing treatments (Figure 3c,d). The more pronounced differences of SOCc were observed at the top 5 cm depth. In this depth, the undisturbed treatment has a greater SOCc by 6 and 16% compared to burning and mowing, respectively (Figure 3c). A similar trend with less variations was observed at the 5–10 cm depth. At 10–20 cm, SOCc did not differ significantly between burning and the undisturbed treatments, but they were significantly higher than mowing. Likewise, SOCs in the 0–5 and 5–10 cm depths were the highest in the undisturbed and the lowest under mowing (Figure 3d). While SOCs showed no significant differences between the treatments at the 10–20 cm, the undisturbed treatment had 8% greater SOC than mowing at the 20–30 cm depth.

Soil Nc and Ns also decreased with the soil depths in all the treatments (Figure 3e,f). In the upper 5 cm, Nc was significantly greater in the undisturbed by 15% than the average of both burning and mowing. At 5–10 cm, Nc was also greater in the undisturbed by 11%

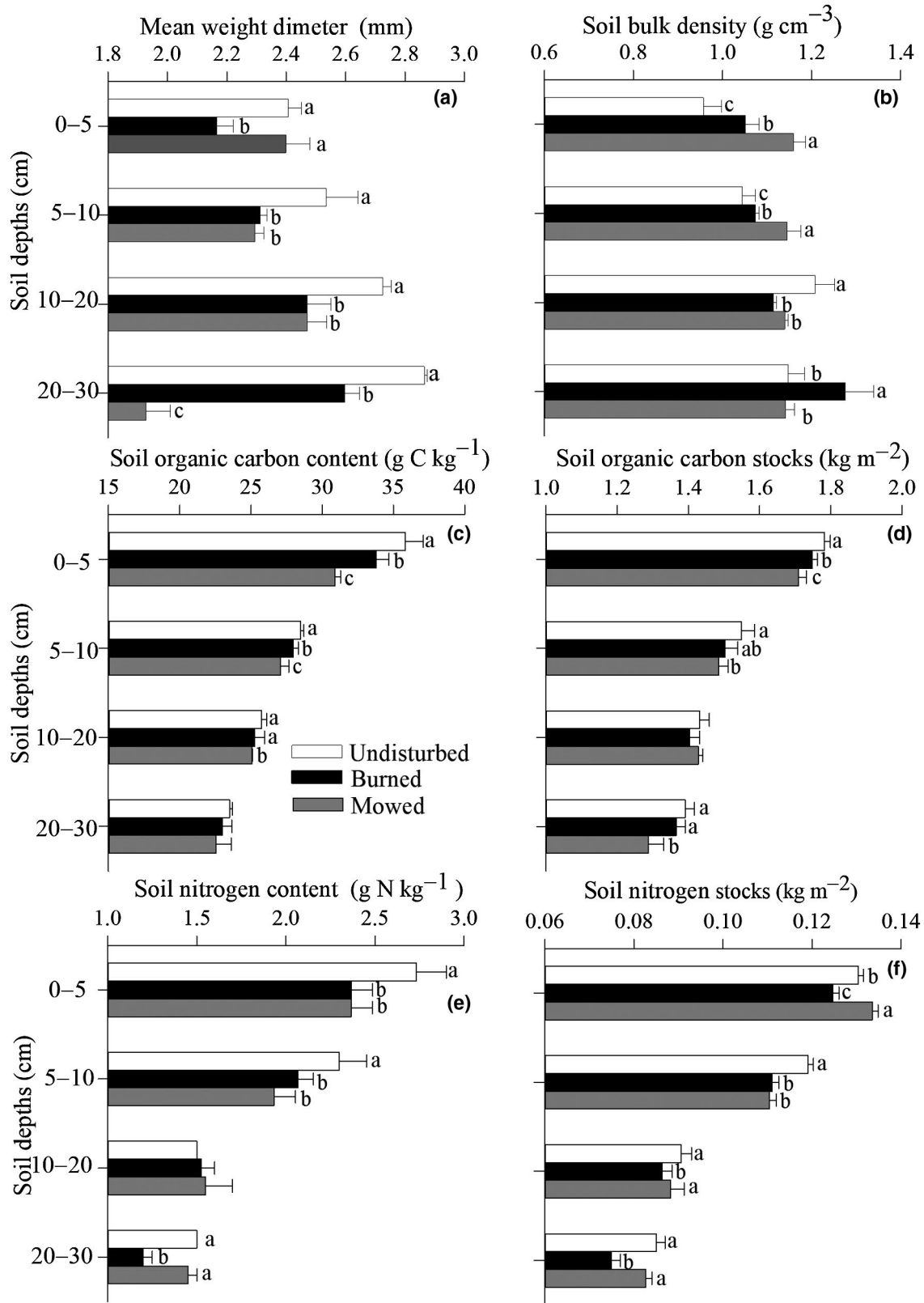


FIGURE 3 Mean ± standard errors of soil properties ([a] mean weight diameter; [b] soil bulk density; [c] soil organic carbon content; [d] soil organic carbon stocks; [e] soil nitrogen content and [f] soil nitrogen stocks) in the four soil depths under annually burned, mowed and undisturbed grasslands. Different letters are significantly different among treatments within a soil layer and aggregate class ($n = 9, p \leq .05$)

and 19% than to the burning and mowing, respectively. While Nc did not differ significantly between the treatments at the 10–20 cm depth, the undisturbed and mowing treatments have greater Nc than burning. Regarding the Ns (Figure 3f), burning had the lowest, and mowing had the highest in the upper 5 cm depth. At the 5–10 cm depth, the highest Ns of $0.12 \pm 0.01 \text{ kg N m}^{-2}$ was observed under the undisturbed treatment. The Ns was significantly lowest in burning at 10–20 and 20–30 cm depths than the undisturbed and mowed grasslands. C:N ratio ranged between 12.39 ± 0.3 observed at 10–20 cm depth under the undisturbed treatment and 19.18 ± 0.32 , recorded under burning at 0–5 cm depth, with burning being the highest in the upper 5 cm (Figure S1).

3.2 | Aggregate size distribution, SOC and N content in the bulk soil fractions

Across the treatments and soil depths, aggregate size distribution (% dry soil) was dominated mainly by macroaggregates (81%), with the large and small macroaggregates representing an average of 39 and 41% dry soil, respectively (Figure 4). The undisturbed treatment promotes the highest proportion of large macroaggregates across soil depths, with burning being intermediate and mowing being the lowest, except for the upper 5 cm depth (Figure 4a–d). At the upper 5 cm depth, the undisturbed treatment had 12% greater large macroaggregates than burning and 7.5% than mowing (Figure 4a). In terms of small macroaggregates, burning had the lowest aggregate size distribution only in the upper 5 cm depth compared to the undisturbed and mowed grasslands. However, below 5 cm depth, burning produced proportionally similar small macroaggregates to the undisturbed and significantly greater than mowing treatment. Burning had the greatest proportion of microaggregates and silt + clay fractions than the undisturbed and mowed grassland in the upper 5 cm depth. The proportion of microaggregates and silt + clay fractions below 5 cm depth decreased in the following order: mowing > burning > undisturbed.

The SOCc in the aggregate fractions was primarily associated with large and small macroaggregates across the treatments and soil depths, with the lowest and highest SOCc associated with the large macroaggregates across the four soil depths in burning and the undisturbed treatment, respectively (Figure 4e–h). For example, burning reduced SOCc in the large macroaggregates by 23% compared to the undisturbed in the upper 5 cm, and this reduction reached 44% at the 20–30 cm depth (Figure 4e). On the other hand, the undisturbed treatment had a higher SOCc associated with small macroaggregates than mowing, except for the 20–30 cm depth where mowing produced 22% greater SOCc in large macroaggregates than the undisturbed grassland (Figure 4h). In contrast to the macroaggregates, grassland burning increased SOCc associated with the microaggregates in the studied depths compared to the undisturbed plots, that is, by 65 and 107% observed in the upper 5 cm and 20–30 cm depths, respectively (Figure 4e,h). The SOCc associated with silt + clay was greater in the undisturbed than burning and mowing

in the upper 5 cm depth. However, the undisturbed grassland induced significantly greater SOCc associated with silt + clay fraction in the 10–20 and 20–30 cm soil depths than the other treatments (Figure 4f,h).

Soil Nc associated with large macroaggregates was largely lower in burning than the undisturbed, with the greatest differences observed at the 10–20 cm (by 73%) and the 20–30 cm (by 161%) depths (Figure 4i–l). The undisturbed treatment also increased Nc associated with the small macroaggregates in the upper 10 cm depth compared to burning and mowing (Figure 4i,j). While grassland burning produced the lowest Nc associated with microaggregates in the upper 5 cm, it promotes the highest in the 10–20 and 20–30 cm, with the undisturbed being the lowest (Figure 4l). The C:N ratio in the whole soil fractions decreased with the increased soil depth in the treatments, with the greatest ratio observed under mowing in the silt + clay and microaggregates below 10 cm depth (Figure S2a–d).

3.3 | Aggregate size distribution, SOC and N content in the large macroaggregates fractions

Within the large macroaggregates, the proportion of aggregates fractions, that is, coarse particulate organic matter (cPOM), microaggregates, silt + clay fractions and their associated SOCc and Nc, differed among the treatments and soil depths (Figure 5). The proportions of aggregates fractions distribution across the treatments were nearly equally distributed between microaggregates (38%), cPOM (30%) and silt + clay (32%) fractions (Figure 5a–d). Grassland burning and mowing induced greater proportions of cPOM within the large macroaggregates than the undisturbed in all soil depths, except for the 10–20 cm layer (Figure 5c). However, mowing induced greater cPOM than burning in the upper 5 cm, but similar values at 5–10 cm and lower proportions below 10 cm depth. The greatest proportion of microaggregates within the large macroaggregates was observed under the undisturbed across the soil depths. The largest variation in the proportion of silt + clay fraction within the large macroaggregates was observed at 5–10 cm depth, where burning decreased the proportion of silt + clay by 51% and 30% compared to the undisturbed and mowed grasses, respectively.

SOCc associated with cPOM fraction occluded in the large macroaggregates was constantly greatest in the undisturbed compared to burning and mowing except at the 5–10 cm layer (Figure 5e–h). For example, at 10–20 cm depth, the undisturbed has a higher SOCc associated with cPOM by 100% ($2.76 \pm 0.67 \text{ g C kg soil}^{-1}$ vs. $5.51 \pm 1.09 \text{ g C kg soil}^{-1}$) to mowing and by as much as 203% to burning ($1.82 \pm 0.71 \text{ g C kg soil}^{-1}$). The SOCc associated with the microaggregates occluded within the large macroaggregates at the upper 5 cm depth showed an opposite trend to that associated with cPOM (Figure 5e). While at the 5–10 cm, burning induced similar SOCc in the microaggregates within the large macroaggregates to the undisturbed (Figure 5f); it significantly increased the SOCc associated with microaggregates at 10–20 and 20–30 cm compared

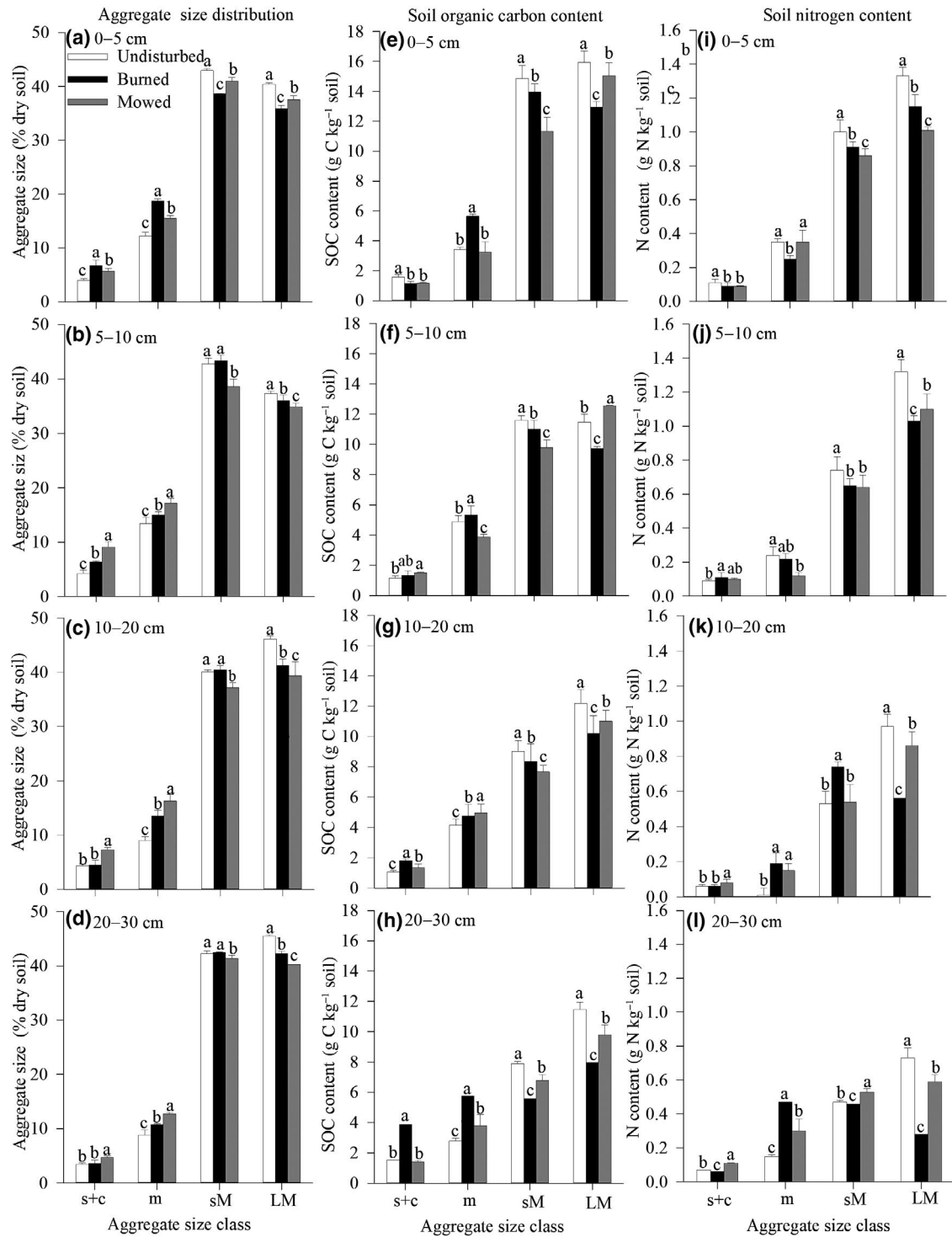


FIGURE 4 Mean ± standard error of the whole soil aggregate fractions (s + c, silt + clay; m, microaggregates, sM, small macroaggregates and LM, large macroaggregates); (a–d) aggregates size distribution; (e–h) soil organic carbon content and (i–l) nitrogen content associated with the fractions in the four soil depths under annually burned, mowed and undisturbed grasslands. Different letters are significantly different among treatments within a soil layer and aggregate class ($n = 9, p \leq .05$)

to the undisturbed and mowed grass (Figure 5g,h). Significant variation in the SOCc associated with the silt + clay within the large macroaggregates observed below 10 cm depth, with burning and the undisturbed grassland being the highest at 10–20 and 20–30 cm, respectively.

Continuous annual burning caused a considerable reduction in Nc associated with cPOM occluded within the large macroaggregates in the four soil depths compared to the undisturbed and mowed grassland (Figure 5i–l). For instance, grassland burning caused 5.6 and 8.8 fold reduction in Nc associated with cPOM

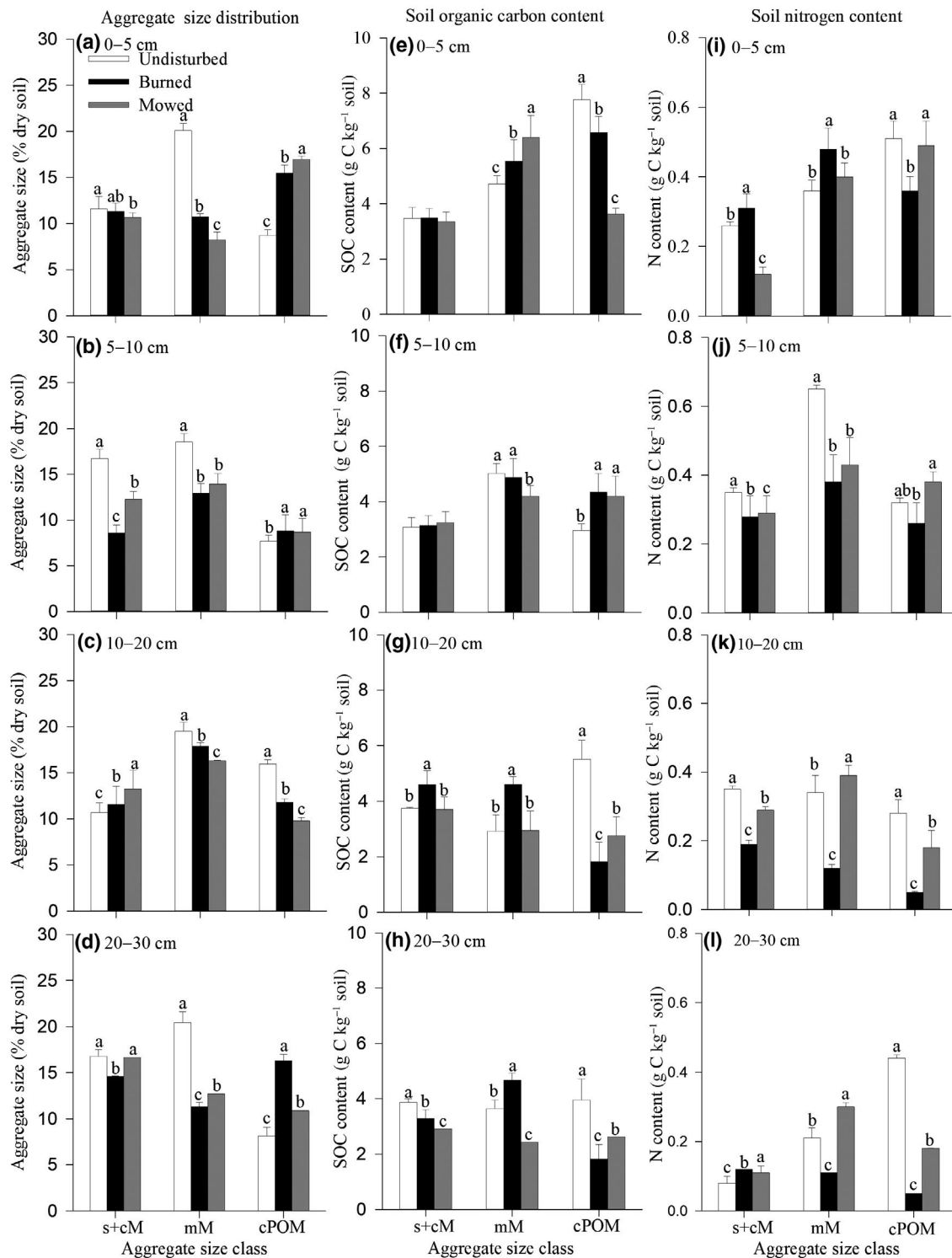


FIGURE 5 Mean \pm standard error of the fractions (s + cM, silt + clay; mM, microaggregates; cPOM coarse particulate organic matter) occluded within the large macroaggregates; (a–d) aggregates size distribution, (e–h) organic carbon content and (i–l) nitrogen content associated with the fractions in the four soil depths under annually burned, mowed and undisturbed grasslands. Different letters are significantly different among treatments within a soil layer and aggregate class ($n = 9$, $p \leq .05$)

occluded in the large macroaggregates at the 10–20 and 20–30 cm compared to the undisturbed, respectively (Figure 5j,k). In contrast, grassland mowing produced greater Nc associated with the microaggregates occluded within the large macroaggregates at 0–5 cm, but the lowest N content in the large macroaggregates at the 5–10 cm and significantly lower

amount below 10 cm depth than the undisturbed. However, burning increased Nc associated with the microaggregates occluded within the large macroaggregates at 0–5 cm, but the lowest N content in the other soil depths compared to the undisturbed with mowing

being intermediate. Burning also caused a significant reduction in Nc associated with microaggregates below 10 cm depth. The Nc associated with silt + clay in the large macroaggregates was the highest in burning and lowest in mowing at 0–5 cm. However, the undisturbed grassland induced the highest Nc associated with silt + clay fraction occluded in the large macroaggregates at 5–10 and 10–20 cm. At 20–30 cm, the undisturbed treatment had the lowest Nc associated with silt + clay compared to the other treatments (Figure 5l).

3.4 | Aggregate size distribution, SOC and N content in the small macroaggregates fractions

Aggregate size distributions within the small macroaggregates were slightly similar to those in the large macroaggregates above the 20 cm depth, with a relatively greater proportion of microaggregates, except for the undisturbed grassland at 20–30 cm (Figure 6). Among the treatments and soil depths, undisturbed treatment induced the greatest proportion of cPOM in the small macroaggregates, with the most considerable differences observed at the 20–30 cm depth (Figure 6a–d). Compared to burning, mowing induced a greater proportion of cPOM within the small macroaggregates at 0–5 cm but lower in the following soil depths. Grassland burning caused the greatest proportion of microaggregates occluded in the small macroaggregates jointly with the undisturbed at the 0–5 cm and with mowing at the 20–30 cm depth. The proportion of silt + clay fractions occluded in the small macroaggregates below 5 cm decreased significantly in the following order; mowing > burning > undisturbed.

Across the treatments and soil depths, SOCc is associated mostly with the cPOM and microaggregates occluded within the small macroaggregates (Figure 6e–h). Generally, the undisturbed treatment induced the greatest SOCc in cPOM within the small macroaggregates except for 10–20 cm, where mowing had the greatest SOCc. Burning surpassed mowing treatment in SOCc associated with cPOM in the small macroaggregates at 0–5 cm and 20–30 cm (Figure 6e,h); however, opposite trends were observed in the other two layers. The SOCc associated with the microaggregates occluded within the small macroaggregates varied significantly above the 20 cm depths.

Similar to SOCc, Nc in the occluded microaggregates within the small macroaggregates associated greatly with the cPOM and microaggregates fractions in the treatments and soil depths (Figure 6i–l). The undisturbed treatment had the greatest Nc associated with the cPOM within the small macroaggregates, except for the 10–20 cm depth. Compared to mowing, annual burning induced high Nc associated with the cPOM below 5 cm (Figure 6i). Nc associated with microaggregates occluded in the small macroaggregates at 0–5 cm was the highest in the undisturbed and lowest in mowing treatment. However, burning induced greater Nc in the microaggregates below 5 cm than the undisturbed, and mowing being the lowest at 5–10 and 10–20 cm (Figure 6j,k). The Nc associated with the silt + clay occluded in the small macroaggregates differs significantly only at 0–5 cm, with a greater amount observed in the burning than the other treatments.

3.5 | Relationship between soil CO₂ fluxes and SOC in the aggregate fractions

Overall, average daily soil CO₂ fluxes in 2014 were significantly greater in burning and mowing by 33 and 16% than the undisturbed treatment, respectively (Figure 7). Soil CO₂ fluxes decreased significantly ($r = -.68$, $p < .001$) with the increase in the stability of soil aggregates and SOCc in the bulk soil (Table 1). However, soil CO₂ fluxes increased significantly with the increase in Nc in the bulk soil. Soil CO₂ fluxes increased with the increased SOCc associated with the large macroaggregates and decreased with the SOCc associated with the silt + clay fractions. Soil CO₂ correlated negatively to cPOM and silt + clay occluded within the large and small macroaggregates, respectively.

4 | DISCUSSION

Overall, investigation of SOC, N content in the soil and aggregate fractions, soil CO₂ emissions, field observation and previous study findings allowed comprehensive illustration of grassland burning effects on SOC dynamics and physical stabilization (Figure 8). Three major mechanisms emerged of the impact of long-term grassland burning on SOC stabilization in the upper 30 cm layer. First, the direct effect of fire on soil surface causing aggregates breakdown and associated C losses (Araya et al., 2017; Mataix-Solera et al., 2011). Second, changing the C input quality and quantity with immediate input after the fire as pyrogenic organic matter alters C and N in the aggregates fractions (Maestrini et al., 2015; Soong & Cotrufo, 2015). Third, the opposite effect of roots and microbial recovery, which enhance new aggregates formation and, at the same time, contribute significantly to the total soil respiration (Pourreza et al., 2014). Worth noting that between the aggregates breakdown and new aggregates formation, substantial C loss might occur by leaching and soil erosion with effect varies depending on soil type, climatic conditions, and fire intensity and frequency.

Sixty-four years of the continuous annual burning of grasslands decreased soil aggregates stability, SOC and N content compared to the undisturbed grassland (Figure 3), suggesting a negative effect of persistent grass burning on soil C and N pools. The trend observed in this study is confirmed by other field and laboratory studies (Girona-García et al., 2018; Jian et al., 2018; Jordán et al., 2011). For example, Jordán et al. (2011) found that high fire severity reduced soil aggregate stability and SOM in volcanic soils under mixed fir and pine forests in Mexico. As hypothesized (first hypothesis), long-term annual burning involves removing aboveground biomass and litter, thus reducing fresh C input to the soil compared to unburned grassland. While fire combusted all the aboveground biomass and litter, mowing does not include removing the surface litter and lower stem leaves (below 5 cm), thus explaining the greater aggregates stability in the upper 5 cm than burning observed in this study (Figure 3a). Surprisingly, the increase in aggregates stability by mowing in the upper 5 cm did not increase SOC and N content

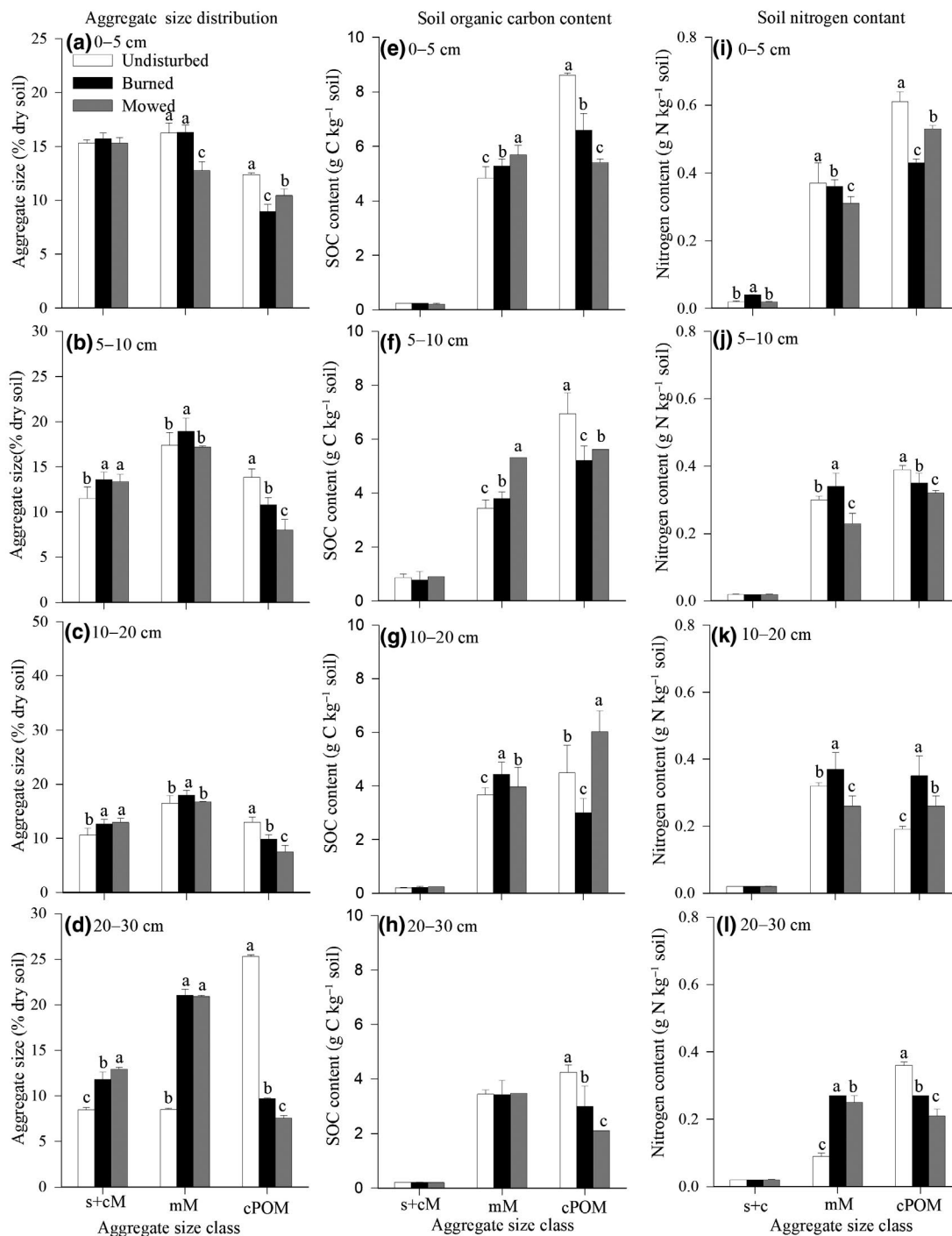


FIGURE 6 Mean \pm standard error of the fractions (s + c, silt + clay; mM, microaggregates; cPOM, coarse particulate organic matter) occluded within small macroaggregate; (a–d) aggregates size distribution, (e–h) organic carbon content and (i–l) nitrogen content associated with the fractions in the four soil depths under annually burned, mowed and undisturbed grasslands. Different letters are significantly different among treatments within a soil layer and aggregate class ($n = 9$, $p \leq .05$)

compared to burning, given the fact that aggregates stability is associated with an increase in SOC and N content in most cases (Goh, 2004; Six et al., 2000; Zhou et al., 2020).

The greater aggregates stability induced by mowing in the upper 5 cm depth than burning might be due to greater root biomass, exudates and turnover under mowing in this layer. Shimoda and Takahashi (2009) reported greater roots biomass and fine roots

distribution in upper 5 cm depth in mowing than burning of grassland soil managed for 17 years in Japan. The current study found greater SOC below 5 cm under annual burning than annual mowing (Figure 3c), suggesting a high impact of both C allocation by roots and the accumulation of pyrogenic organic matter on SOC. Unexpectedly, N content and stocks in the annual mowing at 20–30 cm layer were greater than annual burning (Figure 3e,f), suggesting an enrichment

FIGURE 7 Means \pm standard error of the average daily soil CO₂ emissions in 2014 from soil under long-term annually burned, mowed and undisturbed grasslands. Different letters are significantly different among the treatments ($n = 36$, $p < .05$)

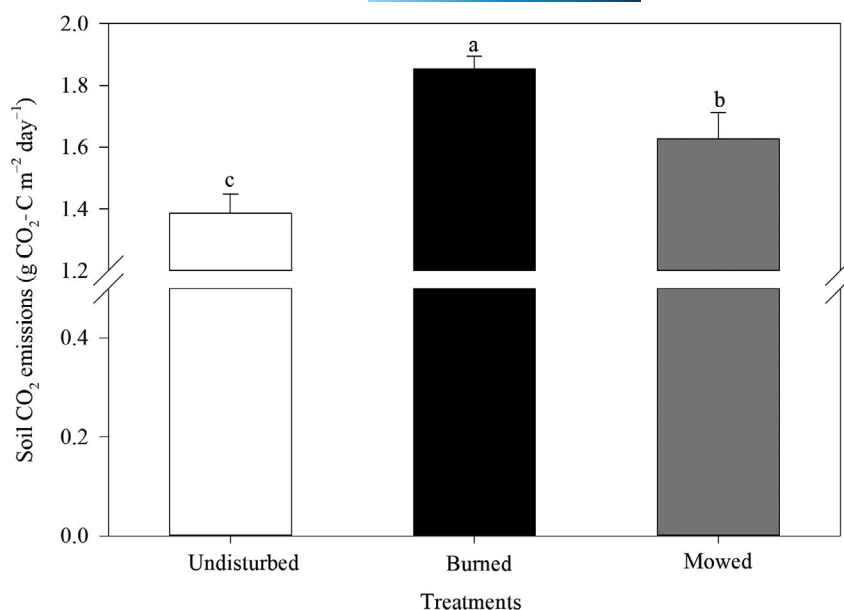


TABLE 1 Pearson's correlation coefficients (r) between soil CO₂ emissions, soil properties (MWD, mean weight diameter; ρ_b , soil bulk density; Nc, nitrogen content and SOCc, soil organic carbon content), SOC associated with the whole soil fractions and with the occluded fractions within the large and small macroaggregates in the upper 10 cm layer ($n = 9$)

	Bulk soil				SOC in whole soil fractions				SOC in LM fractions			SOC in sM fractions		
	MWD	ρ_b	Nc	SOCc	LM	sM	m	s + c	cPOM	mM	s + cM	cPOM	mM	s + cM
CO ₂	-0.68*	0.31	0.68*	-0.35*	0.42*	-0.05	-0.11	-0.42*	-0.61*	0.00	-0.01	-0.06	0.18	-0.35*

*Statistically significant at $p < .05$ level. Whole soil fractions (LM, large macroaggregates; sM, small macroaggregates; m, microaggregates; s + c, silt + clay) and the occluded fractions within the large and small macroaggregates (cPOM, course particulate organic matter; mM, microaggregates; s + cM, silt + clay fractions).

of N relative to SOC by mowing. However, these results were based on pseudo-replication (i.e., one block) due to the shallow soil in the other two blocks.

The undisturbed treatment has the greatest litter retention associated with less to no soil disturbance, thus highly facilitating soil aggregation and accumulation of SOC and N compared to burning. In this experimental site, Fynn et al. (2003) found that surface litter is largely greater in the undisturbed plots by 850% (190 g litter m⁻² vs. 20 g litter m⁻²) than the burned plots. Grassland burning is repeated annually in August, thus not allowing substantial litter incorporation into the soil by fungal and other microbial activities. Additionally, fire highly reduces soil microbes and fungal activities, which require enough time to recover. In a meta-analysis study, Dooley and Treseder (2012) found that fire decreased total microbial biomass and fungal abundance by 33.2, 47.6%, respectively. While soil microbes recovery time after fire still a controversial issue, some studies showed rapid bacterial and slow fungal biomass recovery (Hart et al., 2005; Pourreza et al., 2014). Pourreza et al. (2014) found that fungal recovery time might be extended to more than 1 year after a moderate and severe fire in Zagros oak forest, Iran.

Long-term annual burning decreased the proportion of large macroaggregates and increased the microaggregates compared to

the undisturbed (Figure 4), with similar implications on SOC and N in these aggregates indicating a high fire disaggregation effect, thus confirming the second hypothesis of this study. This effect extended to the small macroaggregates only for the upper 5 cm depth, implying a lower fire effect on the smaller aggregates. Such results suggested a high degradative fire effect by combusting the organic matter that binds the large macroaggregates yielding smaller aggregates size fractions. These findings agreed with other studies from different regions, vegetation types and fire severity (Andreu et al., 2001; Araya et al., 2016, 2017; García-Oliva et al., 1999) and disagreeing with others (Mataix-Solera et al., 2002; Thomaz, 2017). Agreeing with current study findings, Andreu et al. (2002) observed intensive destruction of large macroaggregates (>2 mm) to microaggregates (<0.1 mm) in the upper 5 cm depth affected by the high fire intensity in Mediterranean calcareous soils, Spain. Araya et al. (2017) reported that the C and N concentrations in the larger aggregate size fractions (2–0.25mm) decreased with an increase in temperature so that at 450°C, the remaining C and N were entirely associated with the smaller aggregate size fractions (<0.25 mm) for forest soils in California, USA. However, Mataix-Solera et al. (2002) found that microaggregates (<0.2 mm) were more affected by fire using scanning electron microscopy for soil collected from the Mediterranean environment, Spain.

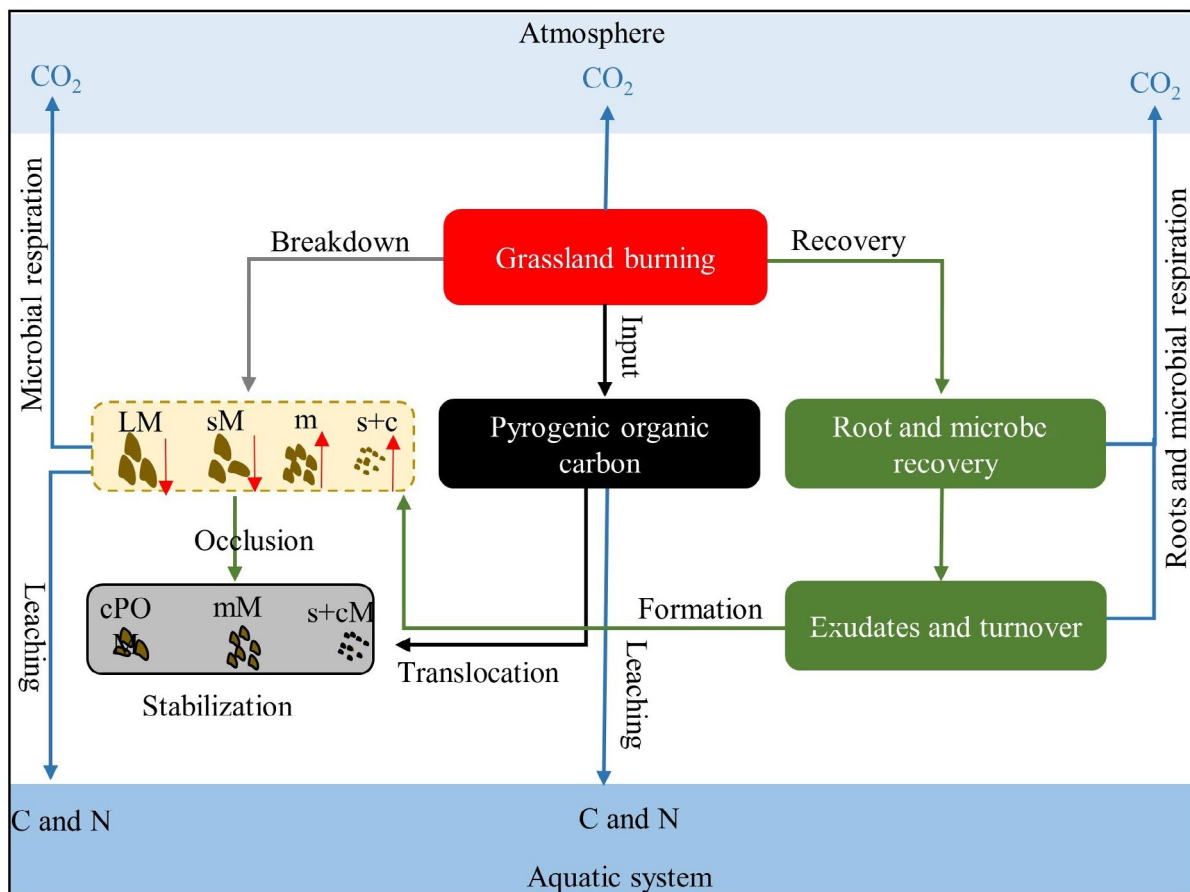


FIGURE 8 Conceptual framework of the effects of grassland burning on soil organic carbon (C) and nitrogen (N) pools and soil-associated processes. The aggregates breakdown, occlusion/stabilization and soil respiration directions observed in the current study, and the other mechanisms, such as C inputs, recovery and translocation, were inferred from previous publications. Whole soil fractions (s + c, silt + clay; m, microaggregates, sM, small macroaggregates and LM, large macroaggregates) and occluded fractions within the macroaggregates (s + cM, silt + clay; mM, microaggregates and cPOM, coarse particulate organic matter)

Annual burning also reduced the proportion of microaggregates occluded within large macroaggregates but still increased the associated SOC across the soil depths (except for the 5–10 cm) compared to the undisturbed treatment (Figure 5), a result found to contradict the second hypothesis expectation. These findings could be explained by the input of the pyrogenic organic matter as a by-product of aboveground biomass burning that partially compensates for the organic matter lost by fire (Mastrolonardo et al., 2015). The partially or totally burned pyrogenic organic matter input may not enhance soil aggregation of previously formed aggregates. Nevertheless, it might increase the SOC within the aggregates due to the fine recalcitrant pyrogenic organic matter accumulation over time and translocation in the aggregates delicate pores. The pyrogenic organic matter has a high aromatic chemical structure and hence is largely resistant to microbial decomposition (Hammes et al., 2006; Hilscher & Knicker, 2011; Soong & Cotrufo, 2015). Therefore, the main loss of the pyrogenic organic matter is caused by translocation inside the soil pore or the transportation to deeper soil horizon or into aquatic systems (Hilscher & Knicker, 2011; Hockaday et al., 2006). The above argument also explains the greater SOC content

associated with microaggregates and silt + clay observed under burning than mowing and the undisturbed treatments in deeper soil depths (below 10 cm) in the whole soil fractions and those occluded within the large and small macroaggregates.

The larger particles (2–0.25 mm) of the pyrogenic organic matter might be included in the occluded fractions within the large macroaggregates and explain, for example, the greater proportion of the cPOM in the large macroaggregates under burning compared to the undisturbed (Figure 5a–d). In addition, the greatest C:N ratio in the occluded fractions within the large macroaggregates (e.g., as much as 37 in the occluded microaggregates) under burning than mowing and undisturbed grasslands (Figure S2) supports such an explanation. In fact, the greatest C:N in the annual burning below the 20 cm depth could primarily be resulting from the accumulation of pyrogenic organic matter as it showed C:N ratio of 33.82 in previous studies (Soong & Cotrufo, 2015). In general, the pyrogenic organic matter found to be added to the soil as particles <2 mm, and mainly (88%) be associated with silt + clay particles (<0.053 mm) in different soil types, Australia (Skjemstad et al., 1996). Brodowski et al. (2006) reported that while the two macroaggregates fractions (large

and small) stored 26% of pyrogenic organic matter (black C), the microaggregates and silt + clay stored almost double this amount (46%) in grassland soils, Germany. It is unknown that the pyrogenic organic matter is preferentially incorporated within the microaggregates or these fractions selectively become enriched with it by specific soil microbes that live inside the microaggregates. Regardless of the mechanisms, the pyrogenic organic matter increases soil aggregation stability, particularly by incorporating C in the microaggregates fractions (Rumpel et al., 2006; Six et al., 2006).

Compared to the undisturbed, burning decreased N content in the cPOM and microaggregates (except at 0–5 cm) fractions occluded within the large macroaggregates fractions (Figure 5), indicating a similar effect of fire on both protected and unprotected soil N. Grassland burning can change N pools in the soil and aggregate fractions, for example, directly by volatilization, pyrolysis and ash deposition process or indirectly through altering soil biotic and abiotic environments (Grogan et al., 2000; Hu et al., 2019; Soong & Cotrufo, 2015). Pathak et al. (2017) found an increase of total N immediately after fire events but a regressive decrease over time in N content within both micro and macroaggregates fractions in degraded Indian grasslands. The immediate increase in N content after the fire was explained by ash deposition (Alexis et al., 2012), while the decrease over time was attributed to soil erosion, surface runoff and leaching (Bodí et al., 2014; Rumpel et al., 2009). In support, Rumpel et al. (2009), in cultivated soil subjected to fire in Senegal, found that 7–55% and 23–46% of initial deposited pyrogenic organic carbon were subjected to horizontal and vertical transport, respectively. On the other hand, the greater N content in both cPOM and microaggregates occluded in the large macroaggregates under the undisturbed and mowed grass could primarily be explained by the continuous fresh C inputs (i.e., litter and roots turnover) associated with non to low soil disturbance. The absence of disturbance under the reference “undisturbed” treatment and the relatively lower disturbance in mowing than burning allows the formulation of macro and microaggregates (Six et al., 2004). Such a trend was observed only in the cPOM within the small macroaggregates (Figure 6), where burning produced lower cPOM size distribution, SOC and N content in most cases than the undisturbed, suggesting different responses of large and small macroaggregates due to the fire, perhaps based on the exposed surface area.

Grassland burning and mowing resulted in higher soil CO₂ fluxes by 33% and 16% compared to the undisturbed, respectively (Figure 7), which was previously assumed to be due to less physical protection of soil C caused by the soil disturbance in these managements (Abdalla et al., 2016). This explanation is confirmed by the current study as the mean weight diameter correlated negatively ($r = -.68$) to the soil CO₂ emissions (Table 1), confirming the third hypothesis of this study. A similar correlation was observed in a positive direction between soil CO₂ emissions and N content, implying a greater effect of N content on soil CO₂ emissions. This is a surprising result since the low N availability in the soil can facilitate SOM decomposition by “N-mining” where soil microbes obtain their N demand leading to a high priming effect, which, in turn, increases

soil microbial respiration (Chen et al., 2014). The current results also showed that soil CO₂ emissions correlated positively to SOC associated with the large macroaggregates and negatively to the one associated with the silt + clay, suggesting that the SOC associated with silt + clay is mainly adsorbed with the clay particles at the mineral phase and therefore resistant to microbial decomposition (Rabbi et al., 2014).

Hence, this study found no correlation between SOC associated with the microaggregates and soil CO₂ emissions; a question might still be raised whether the greater soil CO₂ under burning than the undisturbed emitted due to SOM mineralization or the root respiration. Several studies (e.g. Dooley & Treseder, 2012; Smith et al., 2008; Soong & Cotrufo, 2015) reported that fire could inhibit soil microbial activity and growth. However, roots respiration post-fire increased significantly due to the rapid roots and vegetation recovery (Czimeczik et al., 2006; Singh et al., 2008). The current results on soil respiration are similar to those reported by Muñoz-Rojas et al. (2016) in Western Australia and by Smith et al. (2010) in Canada under different vegetation types. However, both studies indicated that future research should consider separating autotrophic and heterotrophic respiration to fully understand the mechanisms by which fire increases soil respiration. Therefore, being unable to separate the autotrophic and heterotrophic respiration in the current study would be considered one of the study limitations.

Overall, the study results suggested direct links between grassland burning and global warming because of the high C losses from the soil and other greenhouse gases, that is, CO_x, N_xO and CH₄ produced during the anthropogenic biomass burning (Abdalla et al., 2016; Prosperi et al., 2020). According to a recent estimation that considered the small burned areas (<100 ha) for the first time, Ramo et al. (2021) reported that the total burned area in sub-Saharan Africa represents about 70% of the global burned area and is responsible for CO₂ emissions of 1.44 PgC, which is counted for 14% of the global CO₂ emitted from fossil fuel burning. In addition to greenhouse gases, the high nutrients loss shown by the current study might directly affect grassland productivity leading to land degradation that is also found to contribute highly to the greenhouse gas concentration in the atmosphere (Abdalla et al., 2018; Chang et al., 2021). Therefore, the negative implication of annual burning on grassland sustainability and environmental conservation are enormous not only in sub-Saharan Africa but also on the global scale. Some studies, for example, Neary and Leonard (2020), reported that wild and prescribed fires do not significantly affect soil organic C in Mollsol soils because of the large belowground biomass of C in grasslands, savannas and tundras. These effects vary depending on fire frequency, application period, climate, soil and vegetation conditions. However, alternative grassland management such as improved grazing, that is, controlled rotational grazing to limit the negative effect of fire on climate change, is required. Specifically, annual burning did not show potential for grassland rehabilitation compared to high-density short-duration grazing after 2 years of application in a degraded grassland site in the study area (Chaplot et al., 2016).

5 | CONCLUSION

This study investigated soil organic carbon (SOC) and nitrogen (N) dynamics within the soil aggregates after 64 years of continuous annual burning and mowing of grassland site in South Africa. Three main conclusions were drawn from the study results. First, 64 years of the annual burning of grassland reduced soil aggregation and the associated SOC and N within the bulk soil and large macroaggregate fractions compared to the undisturbed grassland encroached with scattered trees. Second, annual mowing decreased soil C losses (e.g., CO₂ emissions) and increased aggregation and the associated C and N content in the soil compared to the annual burning. Third, the non-disturbance of grasslands may offer a strategy to increase grassland potential for C sequestration due to a greater balance between organic matter inputs (i.e., litter and roots turnover) and C outputs. However, the high risk of trees encroachment under undisturbed grasslands, which covered about 50% of the experimental plots in 2014 (field observation), might cause a total loss of grasslands in the near future.

Based on the current study results, mowing can be proposed as a beneficial practice to increase soil C and N content under grassland in the study area. However, it is still not a substitute for burning, particularly in terms of forage quality and quantity (Vermeire et al., 2020). Therefore, future studies need to investigate a combination of burning, mowing and a rest period in one practice to enhance C and N stocks in the soil, increase forage quality and quantity, and protect grasslands from bush encroachment. Another option would be investigating the longer-term performance of rotational grazing, that is, high-density short-duration grazing under different soil conditions, a cost-effective technique that smallholder farmers could easily adopt. Future studies are also required to separate the autotrophic (root-derived CO₂) and heterotrophic (SOC-derived CO₂) respiration using advanced techniques, that is, field isotope labeling approach, under different grassland management, is essential for designing management plans that simultaneously enhance grassland sustainability and environmental conservation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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