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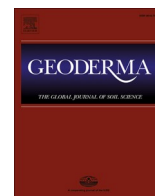
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Inorganic nitrogen and glucose additions alter the short-term formation efficiency of mineral associated organic matter carbon

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ABSTRACT

Carbon within mineral associated organic matter (MAOM) is an important persistent form of soil organic carbon (SOC). However, processes driving the retention of new labile C in MAOM are not fully understood. We investigated the effects of glucose and ammonium nitrate (AN) addition on the short-term (72 h) retention of applied ¹³C-glucose within MAOM. We found an interactive effect of AN addition with the glucose addition rate. Higher rates of glucose addition resulted in proportionally less glucose-C retained, indicating lower MAOM-C formation efficiency. Addition of AN only altered the proportional retention of glucose where glucose was applied at the lowest rate. In this instance glucose-¹³C recovery increased with AN addition. However, after 72 h there was no treatment difference in total MAOM-C, indicating that any changes in formation efficiency as a result of AN and glucose additions, did not result in differences in total MAOM-C in the short-term. Whether and how this affects the medium and longer-term dynamics of MAOM-C requires further investigation.

Increasing soil organic matter (SOM) carbon is a climate change mitigation strategy, which has benefits for a wide range of soil functions (Bossio et al., 2020). Mineral associated organic matter carbon (MAOM-C) is considered to be one of the most persistent forms of soil organic C, composed of low molecular weight (LMW) compounds of plant and microbial origin (Lavalley et al., 2020), and is therefore key to enhancing soil C storage.

MAOM-C is thought to accrue via two distinct pathways, by direct sorption of compounds or via a microbial mediated pathway, in which compounds undergo microbial assimilation, biosynthesis and turnover prior to inclusion with MAOM (Lavalley et al., 2020). Therefore, increased substrate availability is likely to alter the formation of MAOM-C via the microbial formation pathway. Increased microbial growth and turnover can increase MAOM-C due to additional microbial necromass and by-products or result in greater mineralisation of MAOM-C. Greater availability of LMW- organic carbon may result in greater absolute retention of C, but with lower efficiency (i.e. less proportional retention of substrate C in MAOM-C) (Oldfield et al., 2018), due to the emergence of a less efficient, faster growing microbial community (Fierer et al.,

2007). This dynamic poses a challenge for identifying the best C storage strategies.

The application of nitrogen (N) fertilisers is common in agricultural settings, contributing to concentrations of ammonium and nitrate-N in the soil. The additional N helps to alleviate N constraints on plant growth and microbial activity (Gillespie et al., 2014) through facilitating decomposition and production of N-rich microbial by-products, which are important in the formation of MAOM complexes (Kopittke et al., 2020). It may therefore be expected that N addition would increase MAOM-C. However N enrichment can result in decreases in MAOM-C due to soil acidification, and subsequent reduced microbial activity and loss of C preserved by cation bridges (Chen et al., 2020; Ye et al., 2018). Here, we aim to improve the understanding of the short-term dynamics between LMW-C substrate availability and N addition for MAOM-C accrual. We examine the effect of substrate ¹³C-glucose quantity and ammonium nitrate (AN) addition on the short-term (72 h) retention of applied ¹³C within MAOM under controlled environment conditions. Grass exudates are composed of a variety of compounds, influenced by species and their environments (Dietz et al., 2020).

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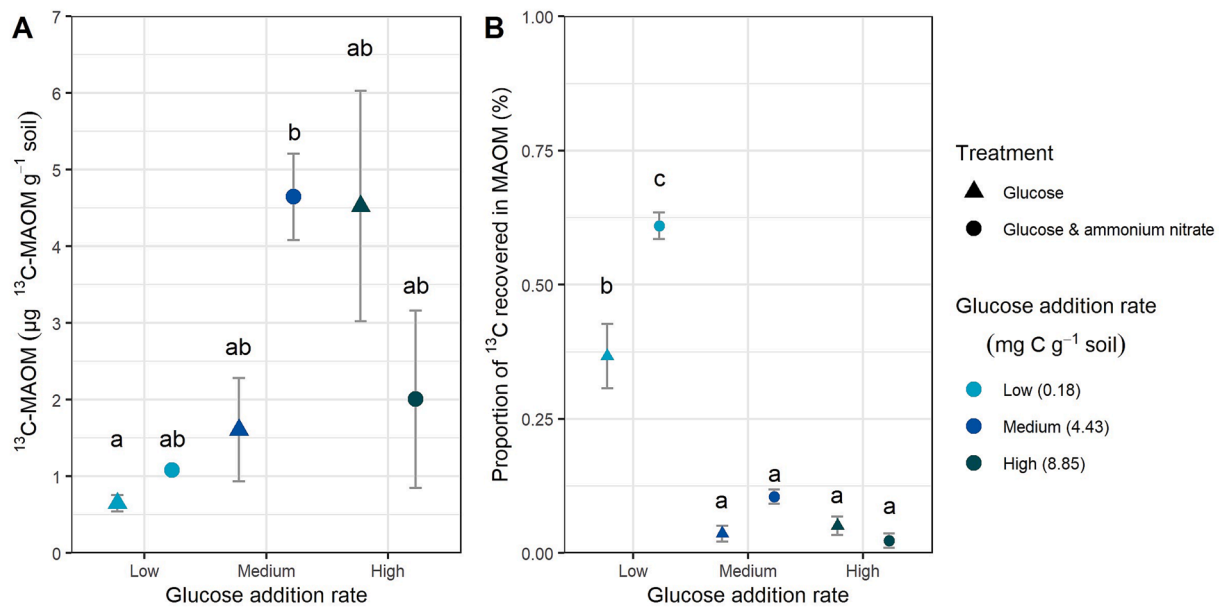


Fig. 1. Absolute substrate-derived ^{13}C recovered in MAOM ($\mu\text{g } ^{13}\text{C-MAOM g}^{-1}$ soil) at 72 h (A), and proportion of applied ^{13}C applied recovered in the MAOM fraction (%) at 72 h (B). The glucose and glucose + ammonium nitrate (AN) treatments are represented as triangles and circles, respectively, for the low (0.18 mg C/g soil), medium (4.43 mg C/g soil) and high (8.85 mg C/g soil) glucose addition rates. Ammonium nitrate addition at 0.28 mg N g^{-1} soil (equivalent to 100 kg N ha^{-1}). Data are means ($n = 3$) and vertical bars indicate \pm one standard error of the mean. Different lowercase letters within each plot represent significant differences between glucose addition rate \times AN treatments ($P < 0.05$).

Carbohydrates are the most abundant compounds in root exudates, with glucose making up 40 – 50 % of this (Gulina and Kuzyakov, 2015). Glucose was used to examine the microbial mediated accumulation of substrate C within MAOM, as it is easily assimilated by multiple taxa (Yuan et al., 2020), and microbial uptake outcompetes direct sorption to mineral surfaces (Fischer et al., 2010). We hypothesised that: i) with increasing ^{13}C -glucose addition more ^{13}C would be recovered in MAOM, ii) the proportion of added glucose C recovered in MAOM, would decrease with increasing rates of addition, and iii) AN addition would increase absolute and proportional ^{13}C recovered in MAOM compared to where no AN was applied.

Uniformly ^{13}C -labelled glucose (99 atom% ^{13}C) was added to soil from a Scottish agricultural grassland (see Supplementary Materials for detailed methodology) at three rates; low (0.18 mg C/g soil), medium (4.43 mg C/g soil) and high (8.85 mg C/g soil), with or without AN addition (0.28 and 0 mg N g^{-1} soil, equivalent to 100 and 0 kg N ha^{-1} , respectively). Carbon controls, with and without AN addition, received deionised water without glucose. Treatments and controls were replicated ($n = 3$) and referred to as: i) control, ii) control + AN, iii) low, iv) low + AN, v) medium, vi) medium + AN, vii) high and viii) high + AN. Core headspace $^{12+13}\text{C-CO}_2$ concentration was determined throughout the incubation period using isotope ratio mass spectrometry. MAOM was isolated by wet sieving through a $53 \mu\text{m}$ sieve, material $< 53 \mu\text{m}$ was classified as MAOM (see supplementary information). The ^{13}C -enrichment of the MAOM fraction, soil pH, and microbial biomass carbon (MBC) were determined at 72 h. Linear mixed models were used to determine the effects of glucose and AN additions on measured soil properties.

Based on a previous study (Oldfield et al., 2018), we hypothesised that higher rate of ^{13}C -glucose addition would result in greater absolute ^{13}C retention in MAOM (i.e., greater $^{13}\text{C-MAOM}$ enrichment). There was a significant interactive effect between glucose and AN addition on $^{13}\text{C-MAOM}$ concentrations ($P < 0.05$, Fig. 1A). For treatments without AN addition, ^{13}C retention in MAOM generally increased with glucose addition rate, with a moderately significant difference between the low and high addition rates ($P < 0.1$). Therefore, increasing ^{13}C -glucose addition did not consistently result in greater $^{13}\text{C-MAOM}$ concentration.

In contrast, ^{13}C retention in MAOM in treatments receiving AN increased from low to medium, but then declined at the highest glucose addition rate. AN addition did not result in consistently higher ^{13}C retention in MAOM within a glucose addition rate (Fig. 1A). We therefore reject our hypothesis i) that AN addition would increase absolute substrate C recovered within each treatment.

The proportional recovery of glucose- ^{13}C in MAOM is indicative of the formation efficiency of MAOM-C. Our original hypotheses ii) and iii) were that the proportional recovery would be higher in the lower C addition treatments, and greater in treatments receiving AN, by alleviating N constraints on MAOM-C accrual through lower C-to-N ratios of inputs. We show a significant interaction between glucose addition rate and AN with regard to the proportion of substrate ^{13}C recovered in the MAOM fraction ($P < 0.05$, Fig. 1B). The low glucose addition rate resulted in the greatest proportional recovery of ^{13}C in MAOM, which was significantly ($P < 0.05$) increased with the addition of AN (C-to-N ratio of the added substrate was 0.6). However, the addition of AN had no effect on proportional ^{13}C recoveries following medium and high glucose application rates, with C-to-N ratios of the added substrates, of 15.8 and 31.6, respectively (Fig. 1B). Glucose addition rate resulted in no difference in MBC or cumulative respiration (Fig. 2A and B). We suggest that the lower proportional $^{13}\text{C-MAOM}$ recoveries following the medium and high glucose addition rates, (both with and without AN) could be due to low C use efficiency (CUE), where proportionally more glucose-C would be used for respiration than for anabolic processes (Islam et al., 2023). Despite higher proportional ^{13}C -retention in the low and low + AN treatments, there was no treatment difference in total MAOM-C concentrations (Fig. 2C), demonstrating that greater MAOM formation efficiency does not necessarily increase total MAOM-C *per se*. It is possible that the applied glucose-C replaced endogenous MAOM-C lost due to priming, resulting in no net gain in MAOM-C. This suggests there is limited capacity to build MAOM-C from glucose additions in this soil. However, it should be noted that it was not possible to fully identify the causes for this. Quantification of ^{13}C -recovery in other SOC pools (e. g. DOC, MBC) and in CO_2 would be required to elucidate the processes leading to the low recovery of substrate ^{13}C into MAOM ($< 0.75 \%$ in MAOM, Fig. 1B).

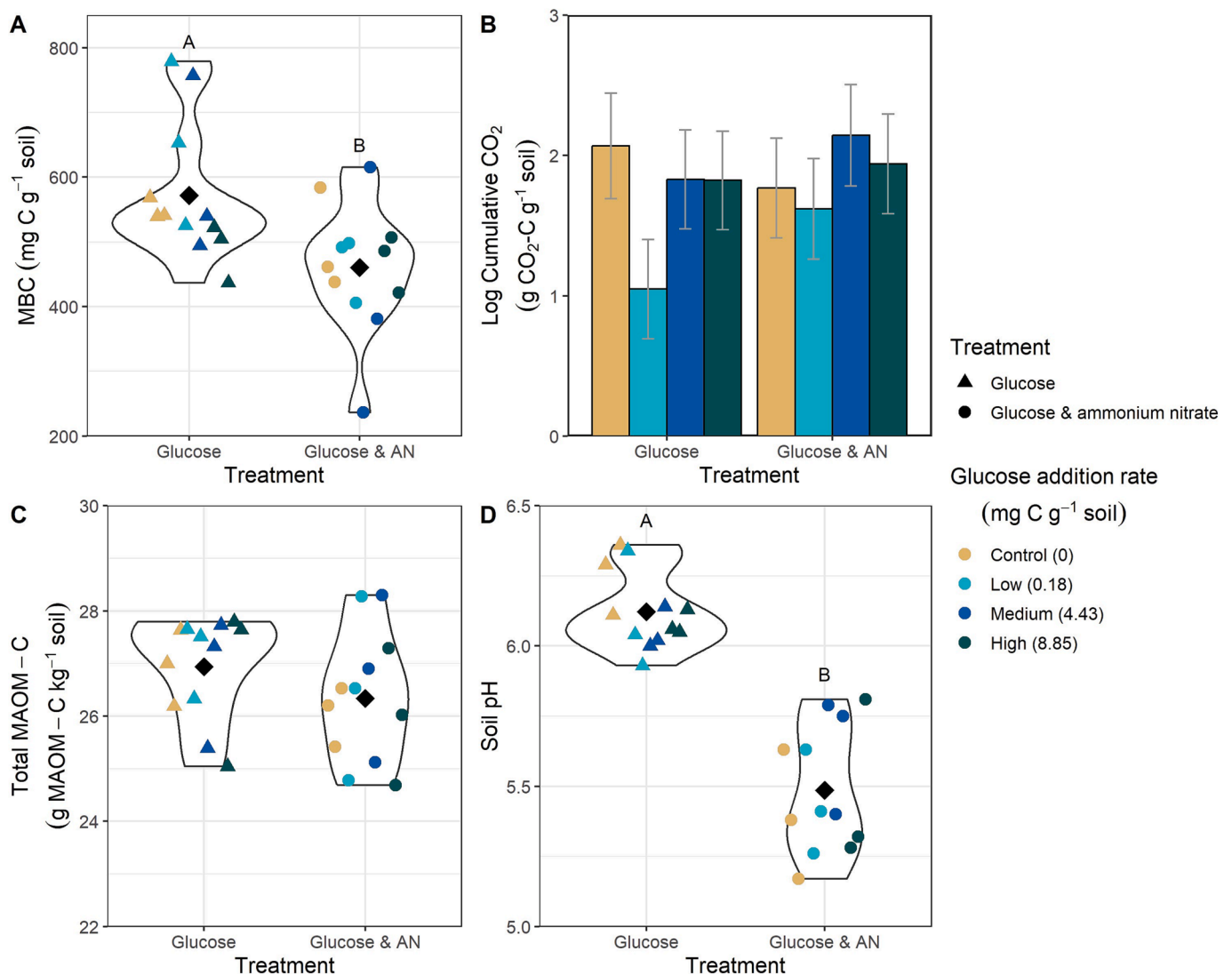


Fig. 2. The effect of glucose addition rate and ammonium nitrate addition on microbial biomass carbon (MBC) (A), cumulative respiration (\log_e transformed) (B), total MAOM-C (C), and soil pH (D) at 72 h. The glucose and glucose + ammonium nitrate (AN) treatments are represented as triangles and circles, respectively for the low (0.18 mg C/g soil), medium (4.43 mg C/g soil) and high (8.85 mg C/g soil) glucose addition rates. Ammonium nitrate addition at 0.28 mg N g^{-1} soil (equivalent to 100 kg N ha^{-1}). In panels A to D, the mean is indicated by a black diamond. Different uppercase letters within each plot represent significant differences between the AN treatments ($P < 0.05$).

Whilst we show a significant interactive effect between AN addition and glucose application rate, AN addition did not result in consistently greater absolute or proportional ^{13}C recovery in MAOM for each glucose rate. Soil pH and MBC concentrations were lower in soils receiving AN than in those not receiving AN across all glucose application rates ($P < 0.001$, Fig. 2A and D, see [supplementary information](#) for detailed pH analysis). Given the speed of pH change after AN addition, it is likely to have resulted from H^+ ions on soil exchange sites being displaced into the soil solution by NH_4^+ rather than any biological processes. There was no significant correlation between soil pH and MBC concentration, ^{13}C -MAOM concentration, or proportional ^{13}C -recovery in MAOM (Fig. S1A to C). It is unlikely that the observed change in pH due to AN addition directly influenced glucose ^{13}C incorporation into MAOM. Instead, we propose that AN addition had an indirect effect on absolute and proportional recovery of glucose ^{13}C in MAOM, mediated by priming of the endogenous SOM. This is in accordance with initial SOM content having a greater influence on retention of input C than the N availability of the input (Castellano et al., 2015; Wu et al., 2022).

Our results showed lower short-term potential formation efficiency of MAOM (proportional recovery of ^{13}C in MAOM) in the medium (4.43

mg C/g soil) and high (8.85 mg C/g soil) treatments, than in the low (0.18 mg C/g soil) treatment. Co-addition of AN (0.28 mg N g^{-1} soil) to the low treatment, resulting in an input C-to-N ratio of 0.63, increased this formation efficiency. This work has provided new insights into the interaction between LMW-C and mineral N addition with respect to short-term MAOM-C formation. However, the implications for long-term MAOM-C persistence are still unknown. For example, increased N availability due to fertilisation, may limit MAOM-C loss due to N mining over longer timescales (Jilling et al., 2018). The effect of N-fertilisation on these processes is important for understanding the dynamics of management practices, SOM priming, and MAOM-C formation and persistence.

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.geoderma.2024.116809>.

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