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RESEARCH ARTICLE

Carbon-to-nitrogen stoichiometry of organic amendments regulates microbial biomass growth and nitrogen mineralization in soil

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Abstract

The carbon-to-nitrogen (C:N) ratio of organic amendments regulates nutrient cycling in soil through its influence on microbial activity. Where an organic amendment has a C:N ratio sparingly higher than that of soil microbes (C:N ratio 6.7—8.7), meeting microbial requirements of carbon and nitrogen may promote microbial biomass growth and nitrogen mineralization. Here we sought to understand the microbial response to the addition of organic amendments with varying C:N ratios and subsequent changes in nitrogen mineralization and plant nitrogen uptake. Dried insect larvae, beef feedlot manure and mixtures of beef feedlot manure with insect larvae or sugarcane residue were used as soil amendments to create a gradient of molar C:N ratios (C_m:N_m) ranging from 6 to 28. An untreated control treatment with a C:N ratio of 17 was also included. Sorghum (*Sorghum bicolor* L.) was grown in control and organic matter amended soils for 20 days after germination. The mixture of beef feedlot manure and insect larvae treatment (C_m:N_m = 9.7) had on average 68% higher microbial biomass compared with the other treatments. Beef feedlot manure with C_m:N_m ratio of 11 did not influence microbial biomass growth. The concentration of mineral nitrogen was highest in the insect larvae (C_m:N_m = 6) treatment, where 23% of the plant nitrogen was derived from the insect larvae as indicated by the $\delta^{15}N$ of plant dry matter. We observed both carbon and nitrogen contents in the organic amendment determine microbial biomass growth and nitrogen mineralization in soil. The results presented here demonstrate that microbial response to organic amendments determines the availability of mineral nitrogen in soil.

KEYWORDS

C:N ratio, feedlot manure, insect larvae, N-acetyl-β-glucosaminidase, nitrogen uptake, β-glucosidase

1 | INTRODUCTION

Globally, agriculture is heavily reliant on a variety of soil amendments—primarily synthetic fertilizers, to support cropping and pasture production (FAO, 2021; Ritchie et al., 2022). Nitrogen fertilizers, such as urea, although convenient to apply, are predisposed to losses through leaching, runoff, volatilization and nitrous oxide

emissions (Bouwman et al., 2002; Davis et al., 2016; Grace et al., 2024; Sebilo et al., 2013). Soil organic matter and organic amendments are significant sources of nitrogen for plants and microbes in agricultural systems (Stevenson, 1982).

Decomposition of soil and added organic matter and mineralization of nutrients by microbes depend on the elemental stoichiometry of C, N, P and S of the organic matter (Coonan et al., 2020; Kirkby et al., 2011; Xu et al., 2015), and most importantly the C to N ratio of the organic matter (Prescott, 2010). Compared with the C:N ratio of agricultural soils (i.e., 11.5; Kopittke et al. (2017)), the C:N ratio of the soil microbes is much lower and can vary between 6.7 and 8.7 (Cleveland & Liptzin, 2007; Coonan et al., 2020). The lower C:N ratio of the microbial biomass than the soil organic matter may suggest that nitrogen available in the soil organic matter is insufficient for microbial biomass growth. Although microbes in soil are primarily carbon limited, the limitation of nitrogen can also affect microbial growth by reducing synthesis of structural proteins and nucleic acids (Soong et al., 2020). Therefore, the C:N ratio of the added organic matter determines microbial biomass growth and mineralization or immobilization of nitrogen.

The mineralization of nitrogen is a microbially mediated process, where microbes utilize the organic carbon and nitrogen for microbial biomass growth, and the nitrogen surplus to microbial demand becomes available to the plants (Ambus et al., 2011). Using global grassland data Risch et al. (2019) have demonstrated a weak positive linear relationship between microbial biomass and nitrogen mineralization in soil. Moreover, the maximum rate of nitrogen mineralization in soil can be as low as 1.09 mg N kg $soil^{-1}$ day^{-1} (Risch et al., 2019). Obviously, the amount of nitrogen available for plant or microbial use through mineralization is dependent on the nitrogen content and chemical recalcitrance of organic matter, in addition to the environmental drivers of the organic matter decomposition in soil (Delgado-Baquerizo et al., 2015; Prescott, 2010; Wang et al., 2004).

The threshold elemental ratio ($TER_{C:N}$) is a useful metric for assessing microbial nitrogen mineralization and immobilization upon addition of organic matter (Sterner & Elser, 2003). Simply, when $TER_{C:N}$ of microbes is lower than C:N ratio of the substrate, nitrogen immobilization takes place and vice versa. Soong et al. (2020) calculated that the average $TER_{C:N}$ of soil microbes is 21, which is greater than the average C:N ratio of soil (~11.5) (Kopittke et al., 2017), poultry manure (~10.1) and vermicompost (~12.2) (Flavel & Murphy, 2006), suggesting net nitrogen mineralization. While the C:N ratio of the common arable crop residues ranging from 20 to 90 (Begum et al., 2014) can impose nitrogen limitation on the microbes and microbial nitrogen immobilization, composted organic manures can provide both carbon and nitrogen for microbial growth and function (Redding et al., 2016). Given the average $TER_{C:N}$, it can be argued that organic amendments with a C:N ratio sparingly higher than the microbial biomass (microbial C:N ratio can vary between 6.8 and 8.7) is better for microbial biomass growth and nitrogen mineralization in soil. However, the carbon utilization efficiency of soil microbes is estimated ~0.3 (Blagodatskaya et al., 2014; Simon et al., 2020; Sinsabaugh et al., 2013), suggesting that the carbon concentration of the organic matter input should be well above what is expected in the microbial biomass, but not exceeding the $TER_{C:N}$ value to avoid nitrogen limitation for microbial growth. To know the upper limit of the C:N ratio of the organic amendment that maximizes microbial biomass growth and nitrogen mineralization, it is necessary to investigate microbial physiological response (i.e., microbial biomass growth, enzyme stoichiometry, enzyme kinetics and community structure) at various levels of C:N ratios of organic amendments.

Indeed, production of extracellular enzymes mediates microbial immobilization and mineralization of the nutrients following '*carbon economy*' principles (Allison et al., 2011). Among the myriads of extracellular enzymes, β-glucosidase and N-acetyl-β-glucosaminidase are considered to be most significantly related to the carbon and nitrogen cycling in the soil (Dick, 2015; Tabatabai et al., 2010). The enzyme β-glucosidase catalyses cellulose, whereas N-acetyl-β-glucosaminidase hydrolyses N-acetyl glucosamine, an amino sugar, which is found in chitin, the fungal cell wall, insect exoskeletons and peptidoglycan of the bacterial cell wall (Moorhead et al., 2016). Sinsabaugh et al. (2008) and Moorhead et al. (2016) suggested that ratios between carbon and nitrogen acquiring enzymes reflect the relative carbon versus nitrogen acquisition activity of the microbes. Understanding the activities of these enzymes may provide clues to the microbial strategy of decomposition and mineralization of the added and soil organic matter.

While enzyme activities can shed light on the microbial response to the organic matter addition in soil, it is important to understand whether the addition of organic matter increases the availability of nutrients through mineralization for growing crops. However, it is difficult to experimentally establish the relationship between nitrogen mineralization of added organic matter and plant uptake because of the complexities of organic matter decomposition. Stable nitrogen isotope (^{15}N) signatures in plants and organic amendments may provide an opportunity to understand the uptake of mineralized nitrogen by plants (Cecchetti et al., 2020; Craine et al., 2015). It has been shown that composted manures, waste feeding insect larvae black soldierfly (*Hermetia illucens* L.) and mealworm (T*enebrio molitor* L.

(Coleoptera: Tenebrionidae)) have stronger ^{15}N enrichment compared with growing plants, which can be utilized to partition the source of plant nitrogen (Hyodo, 2015; Nogués et al., 2023). In addition, dried insect larvae have a nitrogen content of 3%–5% which is higher than composted beef and poultry manure (Amorim et al., 2024). Ecologically, soil is habitat for myriad meso- and macro-fauna belonging to insects (Orgiazzi et al., 2016). The microbial decomposition of chitinous sloughed casts (containing N-acetylglucosamine) of insects can be considered an important source of soil and plant nitrogen.

Here we assessed the influence of organic amendments (composted manure and insect larvae alone or in combination with sugarcane residue) on microbial biomass growth, nitrogen mineralization and plant uptake. The natural abundance of ^{15}N ($\delta^{15}N$) in organic amendments and plant dry matter was used to determine the proportion of amendment-derived nitrogen in plant tissue. We hypothesized that addition of organic amendments with a C:N ratio higher than that of the microbial biomass would promote microbial biomass growth and nitrogen mineralization in soil and subsequent plant nitrogen uptake.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

Pre-germinated sorghum (*Sorghum bicolor* L.) seeds were planted in soil columns amended with composted manure and insect larvae alone or in combination with sugarcane residue. A control treatment was also established, and the plants were grown for 20 days in a growth chamber. The plant growth period was kept short because the mineralization of nitrogen peaks in soil between 10 and 15 days after addition of insect larvae (unpublished lab data). The nitrogen application rate was 80 mg N kg^{-1} (GRDC, 2017) and was added to soil as dry and pulverized composted beef feedlot manure (BM) (C_m:N_m = 11), and insect larvae (BSF) (C_m:N_m = 6). In order to manipulate the C:N ratio of the beef feedlot manure, we mixed milled sugarcane residue (C_m:N_m = 78) or insect larvae in 1: 1 or 13:1 ratio (feedlot manure: sugarcane or insect shell) so that 75% of applied nitrogen came from the feedlot manure. The resulting C_m:N_m ratio of the feedlot-sugarcane residue (BM + CM) mixture was 28, while it was 9.7 for feedlot-insect larvae mixture (BM + BSF). The concentration of carbon, nitrogen and stable isotope composition of the organic amendments is given in Table 1. The organic amendments were mixed with 150 g sieved oxic soil (500–2000 μm) (equivalent to Ferrosol in Australian Soil Classification (Isbell, 2002)) and placed 50 mm below the surface in a 300 mm long and 50 mm diameter soil filled polyvinyl chloride tube lined with a plastic sleeve. Bottom and topsoil layers of the tubes had 665 and 120 g soils, respectively, to give a total soil mass of 935 g. All soil layers received 100 mg kg^{-1} P as K_2HPO4 and 50 mg kg^{-1} S as K_2SO_4. Layers above and below the treatment layer did not receive nitrogen fertilizer. The experiment was laid out in a completely randomized design with four replications of each treatment (beef feedlot manure, BM; insect larvae, BSF; feedlot manure mixed with sugarcane residue, BM + CM; feedlot manure mixed with insect larvae, BM + BSF). We also maintained a control treatment that did not receive any organic amendments. The soil water content was maintained at 70% of the field capacity (soil field capacity was 35%). In each tube a microdialysis probe (MWCO: 20 kDa) (CMA Microdialysis, Kista, Sweden) was inserted diagonally in the fertilizer layer to collect soil solution mineral nitrogen. Each probe was then connected to both a syringe pump (inflow) and fraction collector (outflow) with capillary tubing (CMA Microdialysis, Kista, Sweden). One pre-germinated sorghum (*Sorghum bicolor* L.) seed (MR − Buster, Pacific Seeds™, Queensland, Australia) was planted in each tube, for a total of 20 tubes including all treatments and replicates. The plants were grown at 25°C in a growth chamber for 20 days. The light intensity in the growth chamber was 390 μmol m^{-2} s^{-1} and lights were set for 8 h light day^{-1}. Microdialysis sampling was run on the day of planting and the day before harvest.

TABLE 1 Composition of the soil and organic amendments.

Soil or amendments	C_m:N_m	% Carbon	% Nitrogen	$\delta^{13}C$	$\delta^{15}N$
Oxic soil	17	3.3	0.22	−17.5	7.7
Beef feedlot manure (BM)	11	17.3	1.9	−25.07	19.2
Insect larvae (BSF)	6	41.0	8.0	−23.35	16.18
Sugarcane residue (CM)	78	42.2	0.63	−12.86	7.96
BM + BSF	9.7[a]	19.0	2.3	−24.9[a]	18.44[a]
BM + CM	28[a]	29.7	1.3	−18.97[a]	16.39[a]

[a]Calculated.

For each sampling event, the fraction collectors were set to 150 min collection time at 6°C to ensure samples were kept cool. Previous laboratory work comparing flow rates had determined that 2 μL min^{-1} provided maximum mineral nitrogen (NH_4^+ and NO_3^-) concentration in the outflow solution which was therefore the flow rate used in this experiment. After the 20-day growth period, sorghum seedlings were cut off at the soil surface. Plant dry matter was recorded after drying at 60°C. The soil from each tube was extruded manually by sliding the plastic sleeve out of the PVC column and split into three sections: 0–40, 40–90 and 90–290 mm. The 40–90 mm sample being the treatment layer, which was then analysed for microbial biomass enzyme activities and potassium chloride (KCl) extractable mineral nitrogen (henceforth extractable mineral nitrogen).

2.2 | Microbial biomass

The chloroform fumigation-extraction method was used to determine microbial biomass in soil as outlined in Vance et al. (1987). Briefly, 10 g of moist soil was taken in a glass beaker and placed in a vacuum desiccator. We placed 60 mL of ethanol-free chloroform inside the desiccator and allowed the chloroform to vigorously boil for 2 min under vacuum. The samples were then left in the dark for 24 h. Soon after starting the incubation, the non-fumigated soils were extracted using 0.05 M K_2SO_4 at 1:4 (soil: extractant) ratio (Mehnaz et al., 2019). After 24 h of fumigation, the excess chloroform was removed through repeated evacuation (6-fold) and soil samples were extracted using K_2SO_4. The extracted carbon in non-fumigated and fumigated samples was measured colorimetrically by oxidizing organic carbon with 0.34 M $K_2Cr_2O_7$ and concentrated H_2SO_4 as described in Rayment and Lyons (2011). Following the oxidation reaction, the absorbance of the solution was measured with a Heλios® UV–Visible spectrophotometer at 600 nm (Thermo Fisher Scientific Inc., USA). The microbial biomass carbon (MBC) was estimated using an extraction efficiency factor of 0.38, which was outlined in Vance et al. (1987), and expressed as mg MBC mg SOC^{-1}.

2.3 | Enzyme assays

β-glucosidase and N-acetyl-β-glucosaminidase were selected as the indicator enzymes for decomposition of organic carbon and nitrogen (Sinsabaugh et al., 2008; Tabatabai et al., 2010). L-leucine aminopeptidase may also play a significant role in nitrogen cycling in soil, but its activity is usually lower than N-acetyl-β-glucosaminidase (Sinsabaugh et al., 2008) thus not considered for analysis. The activity of β-glucosidase in soil was measured in Modified Universal Buffer (MUB) (pH 6), using p-nitrophenyl-β-D-glucoside (0.05 M) (PNG) as substrate (Dick, 2015). 0.5 g air-dry sample was weighed into a 50 mL test tube before 2 mL MUB buffer and 0.5 mL PNG substrate were added. The enzyme-substrate reaction was carried out at 37°C for 1 h in a Grant W28 water bath (Grant Instruments, UK). The enzyme-substrate reaction was stopped by adding Tris (hydroxymethyl) aminomethane (THAM) buffer (pH 12). The samples were then filtered with Whatman 41, and the filtrates read on a Heλios® UV–Visible spectrophotometer at 405 nm (Thermo Fisher Scientific Inc., USA). For measuring N-acetyl-β-glucosaminidase activity 0.25 g air-dry soil was taken in 1 mL acetate buffer (pH 5.5), and 0.4 mL p-nitrophenyl-N-acetyl-β-D-glucopyanoside substrate (0.01 M) following the same protocol as described for β-glucosidase (Dick, 2015). We also ran reagent-only controls and soil controls (substrate added in soil just before filtering) to correct the measured enzyme activity (Dick, 2015). Enzyme activity was expressed in μg p-nitrophenol mg SOC^{-1} h^{-1}.

2.4 | Soil mineral nitrogen (NH_4^+-N and NO_3^--N)

Soil solution (collected using micro-dialysis probe) and extractable (1:10 soil to 2 M KCl ratio) mineral nitrogen were determined colorimetrically using Berthelot ($NH_4^+ - N$) and Griess (NO_3^- −N) reactions (Miranda et al., 2001; Rayment & Lyons, 2011; Searle, 1984). The absorbance was measured using a BioTek Epoch 2 microplate spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA).

2.5 | Plant nitrogen uptake and $\delta^{15}N$

The natural abundance of $\delta^{13}C$ and $\delta^{15}N$ in plant material was determined by combustion in a Flash 2000 HT plus Elemental Analyser (Thermo-Fisher Scientific Inc., USA). The combustion products of CO_2 and N_2 (N_2 post reduction) gases were separated and transferred to a Delta V advantage IRMS (Thermo-Fisher Scientific Inc., USA) for isotopic ratio determination. The isotopic ratios were expressed as per mille (‰), relative to the Vienna Pee Dee Belemnite standard for $\delta^{13}C$ and Atmospheric N_2 for $\delta^{15}N$ (Dunn & Carter, 2018), using the following relationship.

$$\delta^{13}C \text{ or } \delta^{15}N\,(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The fraction of plant nitrogen derived from organic amendment (fN_{OA}) was calculated as:

$$fN_{OA} = \frac{{}^{15}\text{NPlant}OA - {}^{15}\text{NPlantcontrol}}{{}^{15}\text{NAmendment} - {}^{15}\text{NPlantcontrol}} \quad (1)$$

where ${}^{15}\text{NPlant}OA$ and ${}^{15}\text{NPlantcontrol}$ are $\delta^{15}N$ signature in plants under organic amendment treatments and control. ${}^{15}\text{NAmendment}$ is the $\delta^{15}N$ signature of the organic amendments.

2.6 | Statistical analyses

Data was analysed using linear mixed effect models to evaluate the effect of treatments on microbial biomass, β-glucosidase, N-acetyl-β-glucosaminidase, β-glucosidase: N-acetyl-β-glucosaminidase, soil solution mineral nitrogen, extractable mineral nitrogen and plant nitrogen uptake using '*nlme*' package (Pinheiro et al., 2016; Zurr et al., 2009) in R (version 4.0.5; R Core Team, 2021). We used spatial variation in light intensity in the growth chamber (varied spatially between 285 and 450 $\mu mol\, m^{-2} s^{-1}$) as random and treatments as fixed effects. The diagnostic plots of the ANOVA models were checked for normality, residual vs. fitted plots and homogeneity of variance (Levene's test). A non-significant Levene's test indicates homogeneity of variance. All model assumptions were met. Post-hoc comparisons of means were performed using the least significant difference tests within the '*emmeans*' package in R (Russell, 2022).

We used constrained redundancy discriminant analysis (RDA) to detect the influence of the explanatory variables (i.e., treatments and C:N ratio of the organic amendments) on the response variables (i.e., microbial biomass, β-glucosidase: N-acetyl-β-glucosaminidase, soil solution mineral nitrogen, extractable mineral nitrogen, and plant nitrogen uptake) in Canoco 5.2 (Microcomputer Power, Ithaca, USA) (Jongman et al., 1995). The statistical significance of the relationships between explanatory and response variables were evaluated using Monte Carlo permutation tests in Canoco (ter Braak & Smilauer, 2012). The association between response and explanatory variables that had p-value <0.05 (adjusted to false discovery rate) was considered statistically significant (Benjamini & Gavrilov, 2009). The RDA ordination was interpreted by biplot rules (Jongman et al., 1995). The arrows of response and explanatory variables indicate the direction in which the values of the variables increase. The cosine of angle between two arrows approximates the correlation between the variables.

We performed structural equation modelling (SEM) using AMOS 24 (IBM SPSS, Amos Development Corporation, Pennsylvania, USA) to evaluate the relationships between microbial biomass, soil solution nitrogen, plant nitrogen uptake, mineral nitrogen and β-glucosidase: N-acetyl-β-glucosaminidase. The multicollinearity between the predictor variables was tested using the variance inflation factor (VIF) in R and the predictor variables did not show any collinearity. The non-significant chi-square (χ^2) test, comparative fit index (CFI) and root mean square error of approximation (RMSEA) were used to find an acceptable SEM model (Schermelleh-Engel et al., 2003).

3 | RESULTS

3.1 | Microbial biomass, activity of β-glucosidase and N-acetyl-β-glucosaminidase

The increase in microbial biomass was on average 1.7-fold higher in the beef feedlot manure and insect larvae mixture (BM + BSF) compared with insect larvae (BSF) and mixture of beef feedlot manure and sugarcane (BM + CM) ($p < .05$) (Figure 1a). The control treatment (C) was also 1.7-fold less than the beef feedlot manure and insect larvae mixture (BM + BSF). However, this difference was statistically non-significant. The microbial biomass in beef feedlot manure (BM), insect larvae (BSF) and mixture of beef feedlot manure and sugarcane residue (BM + CM) treatments were statistically similar. The activity of β-glucosidase in different treatments was also statistically similar (Figure 1b). The activity of N-acetyl-β-glucosaminidase in soils that received a mixture of beef feedlot manure and sugarcane residue (BM + CM) was 51% higher than the insect larvae (BSF) treatment ($p < .05$), whereas differences in N-acetyl-β-glucosaminidase activity in other treatments were non-significant (Figure 1c). The activity ratio of β-glucosidase and N-acetyl-β-glucosaminidase was lowest in the mixture of feedlot manure and sugarcane residue (BM + CM), which was on average two times lower than that of beef feedlot manure (BM) only treatment ($p < .05$; Figure 1d).

3.2 | Soil mineral nitrogen and plant nitrogen uptake

The average concentration of extractable mineral nitrogen was highest in the insect larvae treatment (BSF), which was significantly higher (ca. 1.7-fold) than the mixture of beef feedlot manure and sugarcane residue (BM + CM; $p < .05$; Figure 2a). The mixture of beef feedlot manure with insect larvae (BM + BSF) also had 65% higher extractable mineral nitrogen concentration compared with the

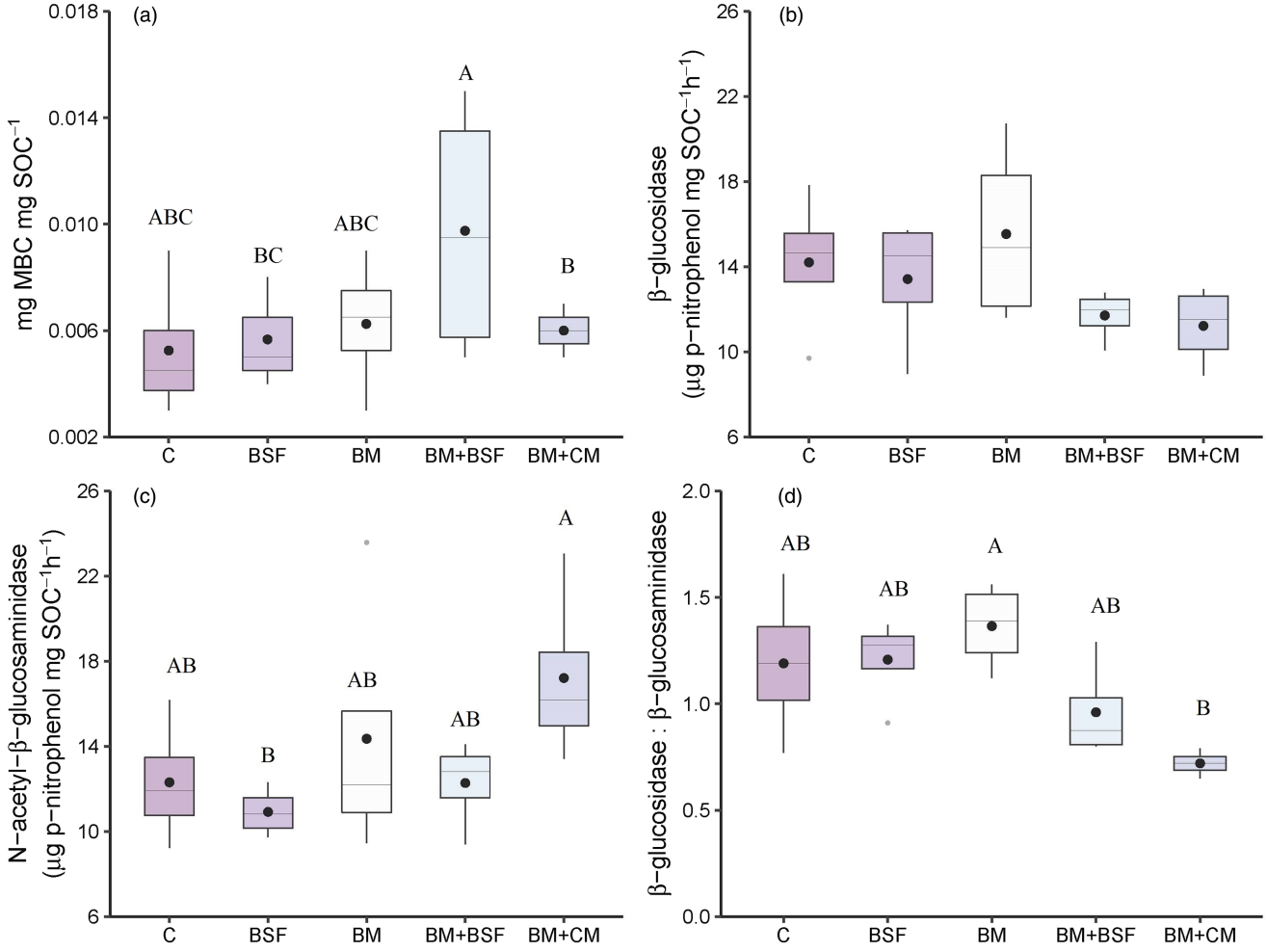

FIGURE 1 Influence of the addition of organic amendments on microbial biomass carbon (a), β-glucosidase (b), N-acetyl-β-glucosaminidase (c), β-glucosidase: N-acetyl-β-glucosaminidase (d). The horizontal lines and black dots inside the box represent medians and means. BM, beef feedlot manure; BSF, insect larvae; C, control; CM, sugarcane residue. Treatment boxes with different uppercase letters are significantly different at $p < .05$.

beef feedlot manure and sugarcane mixture (BM + CM; $p < .05$). The differences in extractable mineral nitrogen concentration in other treatments were statistically non-significant. However, it is worth noting that even the control (C) treatment had 1.4 times more extractable mineral N than the beef feedlot manure and sugarcane residue (BM + CM) mixed treatment. Unlike the variations in extractable mineral nitrogen concentration, the soil solution nitrogen concentration in different treatments was statistically non-significant (Figure 2b). Although there was no statistical difference in plant nitrogen uptake in different treatments (Figure 2c), plant dry matter $\delta^{15}N$ was highest in insect larvae treatment (BSF), which was significantly higher compared with feedlot manure and sugarcane mixture (BM + CM; $p < .05$; Figure 2d). It was estimated that in insect larvae treatment (BSF), 23% of plant nitrogen was derived from the added larvae.

3.3 | Relationship of C:N ratio with microbial biomass, soil solution nitrogen, mineral nitrogen and β-glucosidase: N-acetyl-β-glucosaminidase

The redundancy discrimination analysis (RDA) explained 42% of the total variation ($p < .01$) (Figure 3). Of the explained variations, 21% was explained by C:N ratio of the added organic amendments ($p < .05$) and 13% by the beef feedlot manure and sugarcane mixture treatment ($p < .05$). Using the biplot rule, we observed that the C:N ratio had a strong negative relationship with extractable nitrogen, whereas a positive relationship with plant nitrogen uptake. The structural equation model showed a significant positive relationship of β-glucosidase: N-acetyl-β-glucosaminidase ratio with soil solution mineral nitrogen ($p < .05$) and extractable

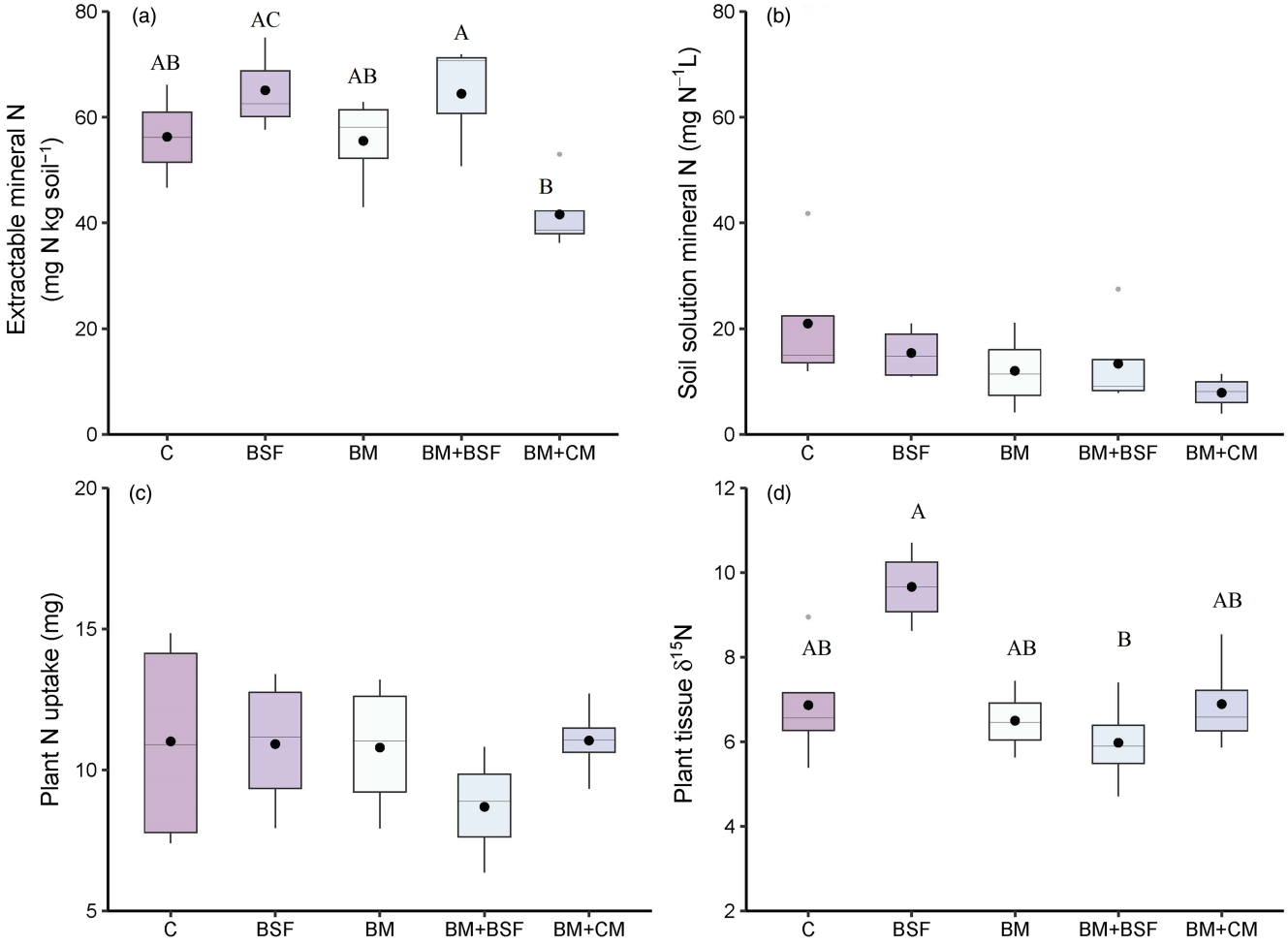

FIGURE 2 Influence of the addition of organic amendments on extractable mineral nitrogen (N) (a), soil solution mineral N (b), plant N uptake (c), plant tissue $\delta^{15}N$ (d). The horizontal lines and black dots inside the box represent medians and means. BSF, insect larvae; BM, beef feedlot manure; C, control; CM, sugarcane residue. Treatment boxes with different uppercase letters are significantly different at $p < .05$.

mineral nitrogen, whereas plant nitrogen uptake had a significant negative relationship with microbial biomass ($p < .05$) (Figure 4).

4 | DISCUSSION

A gradient of C:N ratios was created by using organic amendments alone or in mixture. The prescribed C:N ratios of organic amendments and their mixtures (i.e., 6–28) altered the availability of C and N for microbial utilization (Table 1). According to the $TER_{C:N}$ concept, in this C:N ratio range soil microbes can be either carbon or nitrogen limited (Spohn, 2016; Sterner & Elser, 2003). Conceptually, switching from carbon to nitrogen limitation would reduce microbial biomass growth, change enzyme activity stoichiometry, reduce carbon use efficiency and release excess carbon as carbon dioxide through overflow respiration (Mooshammer et al., 2014; Soong et al., 2020). Here, we demonstrated that the mixture of beef feedlot manure and insect larvae (BM + BSF) with a C:N ratio between microbial biomass C:N ratio and $TER_{C:N}$ (i.e., 9.7) promoted microbial biomass growth and nitrogen mineralization because of the availability of carbon and nitrogen in the amendment that met microbial growth requirements. Organic amendments with a C:N ratio sparingly lower than the microbial biomass C:N ratio (i.e., 6.0, as in BSF) stimulated nitrogen mineralization without an increase in microbial biomass, which suggested inadequate carbon availability in the added amendment. Nevertheless, at this C:N ratio, 23% of plant nitrogen was derived from the organic amendment (Figure 2d). Therefore, an organic amendment with a C:N ratio, which meets microbial carbon and nitrogen requirements for growth, promotes nitrogen mineralization and enhances amendment-derived nitrogen uptake by plants.

Nitrogen content in the beef feedlot manure and insect larvae (BM + BSF) treatment (C_m:N_m = 9.7) was high relative to its carbon content. In fact, the nitrogen content in this mixture was ~2 times higher than the mixture of

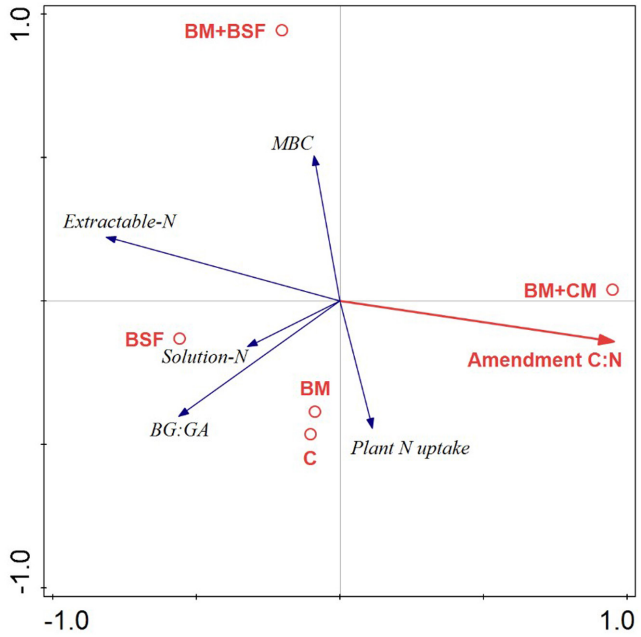

FIGURE 3 Constrained redundancy discrimination analysis (RDA) biplot showing relationships between treatment and C:N ratio of organic amendments with microbial biomass, activity and activity ratio of β-glucosidase and N-acetyl-β-glucosaminidase, soil solution and extractable nitrogen, and plant nitrogen uptake. BM, beef feedlot manure; BSF, insect larvae; C, control; CM, sugarcane residue.

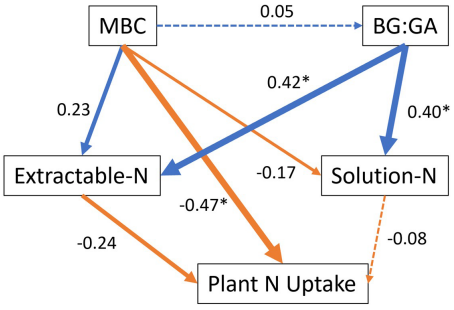

FIGURE 4 Structural equation model showing the relationship between microbial biomass, activity ratio of β-glucosidase: N-acetyl-β-glucosaminidase, soil solution nitrogen (N), extractable nitrogen and plant nitrogen uptake. The arrow heads indicating dependent variables. Solid blue arrows represent positive relationships, and orange negative relationships. The intensity of the arrow indicates strength of the relationship. Numbers adjacent to the arrows are regression coefficients. The level of statistical significance is shown as $*p < .05$, $**p < .01$. $\chi^2 = 0.81$, df = 2, $p < .67$, CFI = 1, RMSEA = 0.00.

beef feedlot manure and sugarcane residue (BM + CM) (Table 1). Additionally, the simple activity ratio of β-glucosidase over N-acetyl-β-glucosaminidase was ~1.0, potentially suggesting sufficient availability of both carbon and nitrogen for microbial utilization (Figure 1d). This observation does not align with the assumption of the $TER_{C:N}$ that the amendment with a C:N ratio of 9.7 should be carbon limited. However, soil microbes are diverse in community structure and carbon use efficiencies. For example, the carbon use efficiency of r-strategists is generally considered lower than that of K-strategists (Andrews & Harris, 1986; Blagodatskaya et al., 2014). Therefore, the $TER_{C:N}$ value proposed by Soong et al. (2020) based on the average values of microbial carbon and nitrogen use efficiencies might not represent the microbial community and microbial carbon use efficiencies in this current experiment. Additionally, rhizodeposition from growing sorghum plants was also a source of labile carbon for the soil microbes (Kuzyakov & Xu, 2013). However, because of the early stage of plant growth, rhizodeposition is expected to be low (Nguyen, 2009). Despite this apparent discrepancy between $TER_{C:N}$ and enzyme activity, carbon content relative to nitrogen might be high enough to trigger microbial biomass growth in BM + BSF.

Compared with the mixture of beef feedlot manure and insect larvae (BM + BSF), in the insect larvae (BSF) (C_m:N_m = 6) treatment microbes had carbon limitations relative to the nitrogen as indicated by $TER_{C:N}$. The simple activity ratio of β-glucosidase over N-acetyl-β-glucosaminidase was >1, which also indicates greater production of carbon acquisition enzyme than the nitrogen acquisition enzyme (Figure 1d). Thus, in nitrogen surplus conditions, the microbial biomass growth might be restricted by carbon limitation. This result corroborates the proposition that a sufficient supply of both carbon and nitrogen is required for microbial biomass growth (Schimel & Weintraub, 2003). Hence, microbial biomass growth was dependent on the C:N ratio of the organic amendments, which, in this current experiment was about 1.4 times higher than the global average of microbial C:N ratio of 6.7 (Coonan et al., 2020). Since the carbon use efficiency of the soil microbes is ~0.3 (Sinsabaugh et al., 2013) and microbes require carbon ($\sim 6 \times 10^{-3}$ mg C mg MBC^{-1} day^{-1}) to maintain cellular structure (Anderson & Domsch, 1985; van Bodegom, 2007), our results support the suggestion that a C:N ratio of an organic amendment higher than the microbial C:N, but not exceeding $TER_{C:N}$ would meet microbial carbon and nitrogen demands and increase microbial biomass. However, the beef feedlot manure-only treatment with a C_m:N_m of 11 did not produce any observable microbial biomass growth (Figure 1a). While beef feedlot manure (BM) had quite a similar C:N ratio to the mixture of beef feedlot manure and insect larvae (BM + BSF), the enzyme activity ratio in the beef feedlot manure (BM) treatment indicated microbial carbon limitation, which might have limited microbial biomass growth (Figure 1d).

Removing carbon and nitrogen limitations in the mixture of beef feedlot and insect larvae treatment (BM + BSF)

also enhanced the mineralization of nitrogen (Figure 2a). It is important to note here that equal quantities of mineral nitrogen were extracted from both the insect larvae (BSF) and beef feedlot and insect larvae (BM + BSF) mixture treatments, suggesting similar nitrogen mineralization (Figure 2a). Therefore, the results we presented here suggest two scenarios that promote nitrogen mineralization upon addition of organic amendments where (i) C:N ratio of organic amendment that supports microbial growth and (ii) organic amendments with C:N ratio lower than that of microbes (Figure 2a).

In contrast, the mixture of beef feedlot manure and sugarcane residue (BM + CM) (C_m:N_m = 28) had carbon in excess, and therefore with this amendment, nitrogen became the limiting nutrient. The assumptions in economic principles of microbial metabolism predict that enzyme production increases when simple nutrient resources are scarce, while the complex ones are abundant (Allison & Vitousek, 2005). This is highlighted within the results of this treatment, where nitrogen limitation, and a carbon surplus triggered greater activity of N-acetyl-β-glucosaminidase compared with β-glucosidase (Figure 1d). In macroeconomics of bacterial growth theory, Koch (1985) argued that enzyme production should be linked with greater resource acquisition. While the activity of enzymes linked with decomposition of nitrogen-containing compounds increased in this treatment, it did not produce an increase in microbial biomass, which might suggest microbial carbon investment in enzymes to decompose nitrogen-containing amino sugars restricted microbial biomass growth (Malik et al., 2020; Ramin & Allison, 2019; Figure 1a).

The negative relationships presented in the RDA biplot between the C:N ratio of the organic amendments and the extractable and soil solution mineral nitrogen align with the view that microbial carbon limitation and availability of nitrogen in the amendments produced mineral nitrogen surplus to microbial requirements (Figure 3). This was evident through the high concentration of soil solution and extractable mineral nitrogen in the insect larvae treatment (C_m:N_m = 6) (Figure 2a,b). Complex nitrogen-containing substrates are not available for microbial use, whereas enzymatically cleaved or depolymerized substances like amino acids or amino sugars are suitable for microbial utilization (Robertson & Groffman, 2015). As Schimel and Bennett (2004) explained, microbes would be less nitrogen limited when a nitrogen-rich substrate is added, and they can mineralize enzymatically depolymerized nitrogen compounds. The relationships in the SEM also demonstrated that both extractable and soil solution mineral nitrogen had significant positive relationships with the enzyme activity ratio of β-glucosidase and N-acetyl-β-glucosaminidase (Figure 4). Such relationships might also imply that microbial carbon limitation regulates the nitrogen mineralization in soil (Manzoni et al., 2012; Mooshammer et al., 2014). For example, nitrogen content in the insect larvae was high (~80 mg N g insect larvae^{-1}) and the ratio between β-glucosidase and N-acetyl-β-glucosaminidase was also >1, which suggests high carbon demand facilitated nitrogen mineralization without increasing microbial biomass (Table 1 and Figure 1a,d). Although nitrogen mineralization was not solely dependent on the activity of enzymes we measured in this current study, the involvement of other enzymes within the nitrogen cycle is capable of mineralizing nitrogen from the soil and added organic amendments or depolymerized compounds (Burns, 1982; Dick, 2015; Gianfreda & Ruggiero, 2006). The experimental evidence we presented here clearly demonstrated the capacity of soil microbes to modulate metabolic pathways to carbon and nitrogen availability (Serbanescu et al., 2020; Soong et al., 2020). We did not analyse $\delta^{13}C$ of CO_2 emission and microbial biomass carbon and therefore, it is not possible to ascertain what proportion of soil organic matter was utilized by the microbes.

It is important to note here we observed a significant negative relationship between plant nitrogen uptake and microbial biomass (Figures 3 and 4). Nitrogen requirements of the sorghum seedling at the early stage of growth (20–30 days after germination) are approximately 20–25 kg N ha^{-1} (Pacific Seeds, 2019). The extractable mineral nitrogen concentration in the soil was almost double what was required by the plant (Figure 2a). Hence, plant uptake cannot be linked to the unavailability of soil nitrogen or to competition between plant and microbes for nitrogen acquisition (Kuzyakov & Xu, 2013; Zhang et al., 2023), at least at an early stage of plant growth. Consequently, because of low plant nitrogen demand, our results did not exhibit any statistically significant relationship between plant uptake and mineral nitrogen in soil (Figure 4). Despite the non-significant relationship between plant uptake and extractable mineral nitrogen, at treatment level based on $\delta^{15}N$ signature in the plant, 23% of plant nitrogen was derived from added amendments in the insect larvae treatment, where the concentration of extractable mineral nitrogen was also high (Figure 3). The $\delta^{15}N$ signature in other treatments did not vary significantly from the control, which might suggest non-traceable uptake of amendment-derived mineralized nitrogen (Figure 2d). During organic nitrogen transformation by microbes in soil, isotopic fractionation of nitrogen is likely to occur (Evans, 2007). Microbial decomposition of ^{15}N enriched organic substrate may release ^{15}N depleted mineral nitrogen in soil and retain ^{15}N enriched compounds in the microbial biomass (Soldatova et al., 2024;

Wynn et al., 2005). The mineralized nitrogen though can be repeatedly utilized by microbes and may undergo multiple fractionation steps (Cui et al., 2020; Evans, 2007), which could restrict our ability to trace the transfer of ^{15}N enriched organic amendment-derived nitrogen to the plants. Further work on $\delta^{13}C$ and $\delta^{15}N$ in the microbial biomass is warranted to better understand the microbial utilization and mineralization of the added organic amendments.

This experiment demonstrated a means of manipulating the C:N ratio of manure with the novel use of insect larvae to improve nitrogen mineralization and microbial growth in soil. Our results also show that insect larvae alone increased nitrogen mineralization, as well as nitrogen uptake by growing plants, which underpins the potential use of insect-derived products to alleviate or supplement plant nitrogen demand.

5 | CONCLUSION

Results of the current experiment clearly demonstrate that carbon and nitrogen contents in the added amendment regulate microbial biomass growth and nitrogen mineralization. The $\delta^{15}N$ signature of the plant dry matter provided limited evidence of the uptake of organic amendment-derived mineral nitrogen by the plants. However, the data we presented here is simple and gives us a cursory view of biogeochemical processes that take place after the addition of organic amendments. To better understand the microbial utilization and release/mineralization of added organic amendments, we need to identify the temporal variation in microbial community, microbial biomass turnover rate and microbial utilization efficiency of the added carbon. Integrating microbial physiological responses, nitrogen mineralization and priming of soil carbon would be powerful to mechanistically explain nitrogen release and carbon stabilization in organic matter-amended soils.

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DATA AVAILABILITY STATEMENT

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

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