1 **Differential vulnerability of mineral-associated and particulate soil organic**

2 **carbon to nitrogen addition in a subtropical forest**

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Abstract

Nitrogen (N) deposition rates of terrestrial ecosystems have gradually declined but are still high in some areas, and previous studies have reported that N addition elicits diverse impacts on soil organic carbon (SOC) pools. SOC can be divided into different functional fractions, namely particulate organic carbon (POC) and mineral-associated organic carbon (MAOC). The responses of these fractions to N addition should be elucidated to better understand the changes in SOC pools. We investigated the effects of N addition treatments (+0, +40, and +80 kg N ha^{-1} yr^{-1}) in a subtropical *Castanopsis fabri* forest to simulate N deposition. The surface (0-10 cm) SOC content in different fractions, aboveground litter biomass, fine root (diameter < 2 mm) biomass, soil exchangeable cation content, and soil enzyme activity under different N addition treatments were measured. The results showed that 1) N addition exerted a positive effect on POC content but did not significantly affect MAOC content. 2) POC content was negatively correlated with pH and soil enzyme activity and positively correlated with aboveground litter biomass, suggesting that POC accumulation was influenced by aboveground litter input and microbial decomposition. 3) Root biomass was unaffected significantly by the addition of N, which could be responsible for the limited response of MAOC to N addition. Furthermore, a close negative relationship was observed between exchangeable Al^{3+} and Ca^{2+} or K^{+} contents, indicating the presence of a trade-off between negative effects of exchangeable cations on SOC bridging and their positive effects on SOC adsorption, thus resulting in an insignificant reaction of MAOC to N addition. Overall, N addition reduces the persistence and increases the nutrient density of SOC, and MAOC with more protection is less vulnerable to N addition than POC with less protection. By incorporating these nuances into ecosystem models, it is possible to more accurately predict SOC dynamics in response to global change.

Keywords: carbon persistence; exchangeable cations; nitrogen addition; soil organic carbon fractions

1 Introduction

50

51 As the biggest carbon (C) sink in terrestrial ecosystems, the soil organic carbon

52 (SOC) pool is essential to the C cycle-climate feedback (Bradford et al., 2016). C and

53 nitrogen (N) cycles are considered tightly linked (Greaver et al., 2016; Cheng et al.,

54 2019). Increased atmospheric N deposition resulting from excessive agricultural,

55 industrial, and urban development has adversely affected SOC quality and ecological

56 diversity in recent decades. This phenomenon has garnered notable attention from the

57 scientific community concerning its impact on SOC pool (Clark and Tilman, 2008).

58 Although the N deposition rate has gradually stabilized, it is higher in southeast China

59 than in other regions within the country (Yu et al., 2019). Several researches have

60 examined the effects of N addition on SOC and have reported that SOC pool may rise,

61 fall, or remain unchanged with N addition (Li et al., 2022; Wang et al., 2023; Wu et al.,

62 2023); overall, there is no unified conclusion on the effect of adding N on SOC.

63 Changes in plant C supply, SOC decomposition, microbial turnover, and

64 interactions between SOC and soil minerals all may affect the response of SOC to N

65 addition (Ye et al., 2018; Chen et al., 2021). Complex SOC composition may be the

66 reason for the varied responses of SOC to N addition (Six et al., 2002; Lugato et al.,

67 2021). Recently, the theory that the chemical complexity of SOC primarily governs its

68 persistence has been criticized for lacking thermodynamic evidence and for neglecting

69 microbial accessibility to substrate components (Schmidt et al., 2011). On the contrary,

70 the mineral-aggregate protective mechanism is considered as an acceptable explanation

71 for SOC persistence. Based on particle size widely used in SOC fractionation, SOC can

72 be divided into particulate organic carbon (POC, >53 μm) and mineral-associated

73 organic carbon (MAOC, <53 μm) (Lavallee et al., 2020). Dividing SOC into different

74 functional fractions may better elucidate SOC dynamics under N addition (Chen et al.,

75 2020; Lavallee et al., 2020). The majority of POC consists of plant components that are

76 readily decomposable (i.e., carbohydrates and aliphatic compounds), and it has a faster

77 turnover rate (Witzgall et al., 2021). MAOC, is primarily made up of low-molecular-

78   weight compounds (i.e., root exudates) and is shielded from breakdown by the chemical

79   bonds between SOC and minerals, as well as microaggregates; thus, MAOC has a

80   slower turnover rate (Poeplau et al., 2018) and is relatively stable (Lavallee et al., 2020).

81   Acidification is a common reaction of soil to N addition because of the 1) release

82   of H^+ during nitrification, 2) release of H^+ after plant uptake of NH_4^+-N, and 3) leaching

83   of exchangeable cations (Tian and Niu, 2015). The different functional fractions of SOC

84   are susceptible to soil acidification. For instance, Rocci et al. (2021) elucidated that N

85   addition resulted in increased POC and MAOC levels, and attributed the accumulation

86   of POC to soil acidification-induced reduction in microbial biomass and SOC

87   decomposition rate (Riggs and Hobbie, 2016). Moreover, N addition can enhance plant

88   productivity (Feng et al., 2023), which may promote POC accumulation. According to

89   Angst et al. (2021), about 60% of MAOC comes from plants, and plant-derived

90   compounds are directly adsorbed onto soil minerals or aggregates (Craig et al., 2022),

91   which promotes MAOC formation. Conversely, studies have shown adverse effects of

92   N addition on POC and MAOC levels (Ye et al., 2018; Zhang et al., 2019; Chen et al.,

93   2020). Guo et al. (2010) proposed that N addition reduced the production of microbial

94   necromass and did not support the stable soil aggregate formation. Therefore, in the

95   absence of physical barrier protection, POC is more easily decomposed by

96   microorganisms (Lehmann and Kleber, 2015), resulting in a reduction in POC content.

97   The size of MAOC pool is determined by the equilibrium between adsorption and

98   desorption rates of charged components (Wu et al., 2023). The fast leaching of cations

99   (e.g., Ca^{2+}) brought on by N addition changed the adsorption rate of C compounds on

100   mineral surface, which was not conducive for the formation of MAOC, thus affecting

101   the persistence of SOC (Ye et al., 2018). Therefore, the mechanism of how POC and

102   MAOC react to N addition has not yet been determined.

103   We carried out a N addition experiment in a subtropical *Castanopsis fabri* forest

104   to investigate the effects of N addition on POC and MAOC as well as their underlying

105   mechanisms. The SOC fractions and various parameters, including aboveground litter

106 biomass, fine root biomass, soil enzyme activities, soil physicochemical properties, and

107 exchangeable cations were examined. Based on previous findings, we proposed two

108 hypotheses: 1) N addition promotes plant biomass and enhances C input, which may

109 promote POC accumulation; 2) N addition causes the loss of exchangeable cations (e.g.,

110 Ca^{2+}) through leaching, affecting the bridge between SOC and polyvalent cations,

111 which may decrease MAOC content.

112

2 Materials and methods

113

2.1 Study site

114

115 Daiyun Mountain National Nature Reserve in southern China's has been selected

116 as an experimental area (25.64°−25.73° N, 118.09°−118.34° E, 1800 m a.s.l.), which

117 has a typical subtropical oceanic monsoon climate. The reserve's average annual

118 temperature and rainfall are roughly 17.6 °C and 1700–2000 mm, respectively, and

119 precipitation mainly occurs during March–September. The dominant tree species are

120 *Castanopsis fabri*. The understory vegetation includes *Micheliamaudiae*, *Lindera*

121 *aggregate*, *Schima superba*, and *Ilex pubescens*. The soil type is Ultisol according to

122 US Department of Agriculture soil order, with 45% sand, 33% silt, and 22% clay.

123

2.2 Experimental design

124

125 Based on the rate of atmospheric N deposition in the area reported earlier, we

126 selected the following three treatments: control (CT, +0 kg N ha^{-1} yr^{-1}), low-N addition

127 (LN, +40 kg N ha^{-1} yr^{-1}), and high-N addition (HN, +80 kg N ha^{-1} yr^{-1}). Each treatment

128 has four plots or replicates, with each plot measuring 10 m × 10 m and having a 10-m

129 buffer zone between them. Beginning in early May 2020, urea (CO(NH$_2$)$_2$) was

130 consistently sprayed over the low-N addition and high-N addition plots using a

131 backpack sprayer after being dissolved in deionized water. On the control plots, the

132 same volume of deionized water was sprayed. We did not observe any marked

133 differences between basic physicochemical properties of the soils of different plots

134 before N addition. In December 2021, we used the five-point sampling method to

135 sample soil from each plot. The surface litter was removed, and the topsoil (0–10 cm)

136 was collected from each plot. Each plot's samples were thoroughly combined, placed

137 in a low-temperature insulated box, and taken right away to the lab for further

138 processing. In the lab, the soil was sieved through a 2-mm screen after being freed of

139 fine gravel, trash, and plant roots using tweezers. The sieved soil was partially stored

140 in a 4 °C refrigerator. Within one week, soil enzyme activity, microbial biomass,

141 mineral N were evaluated. The other soil was air-dried and the SOC fractions and soil

142 pH were examined. To quantify the soil physicochemical characteristics and

143 exchangeable cations (Table S1), a piece of the air-dried soil was filtered through a

144 mesh of 0.149 mm.

145

146 **2.3 Soil physicochemical characteristics**

147 Soil pH was measured using a glass electrode (soil: water; 1: 2.5) (Starter 300,

148 USA). After inorganic C was eliminated from the soil samples using 0.5 mol L^{-1} HCl,

149 SOC and total N (TN) contents were measured using a CN analyzer (Elementar Vario

150 EL III, Germany). According to Vance et al. (1987), the chloroform fumigation-

151 extraction method was used to measure the amount of microbial biomass carbon (MBC).

152 Using a continuous flow on auto-analyzer (SKALAR San++, Netherlands), soil mineral

153 N was extracted in a 2 mol L^{-1} KCl solution and quantified.

154

155 **2.4 SOC fractions**

156 SOC fractions were investigated using the physical fractionation (wet sieving)

157 method described by Marriott and Wander (2006). Specifically, we weighed 20.0 g of

158 dried soil samples into plastic bottles, and added 100 mL of sodium hexametaphosphate

159 solution (5 g L^{-1}) to each sample. The mixture was first shaken for 18 h. Subsequently,

160 the dispersion was transferred to a 53-μm sieve of a reciprocating shaker (AS200

161 control, Germany) with an amplitude of 3 cm, and the samples were subjected to 100

162   vibrations. A continuous flow of deionized water was used to flush the sieve to disperse

163   the SOC within aggregates. Meanwhile, a clean beaker was used to collect the sample

164   passing through the 53-μm sieve. Soil samples collected on the sieve and in the beaker

165   were dried at 60 °C until their weight remained stable. The SOC content in each fraction

166   was evaluated using an elemental analyzer (Elementar Vario EL III, Germany). The

167   following equations were used to obtain the POC (g kg^{-1}) and MAOC (g kg^{-1}) contents:

168   $$\text{Mass recovery (\%)} = \frac{\text{Mass}_{POC} + \text{Mass}_{MAOC}}{\text{Mass}_{Bulk\ soi}} \times 100 \qquad [1]$$

169   $$\text{POC (g kg}^{-1}) = \frac{(\text{Mass}_{POC} \times OC_{MAOC})}{\text{Mass}_{Bulk\ soi} \times \text{Mass recovery}/100} \qquad [2]$$

170   $$\text{MAOC (g kg}^{-1}) = \frac{(Mass_{MAOC} \times OC_{MAOC})}{Mass_{Bulk\ soi} \times \text{Mass recovery}/100} \qquad [3]$$

171   where Mass$_{Bulk}$ is the soil mass used for sieving (g), Mass$_{POC}$ and Mass$_{MAOC}$ are the

172   masses of the POC and MAOC fractions obtained after sieving (g), and OC$_{POC}$ and

173   OC$_{MAOC}$ are the C contents of POC and MAOC (g C kg^{-1} fraction), respectively.

174

175   **2.5 Exchangeable cations and soil enzyme activities**

176   Soil exchangeable cations (K$^+$, Na$^+$, Ca^{2+}, Mg^{2+}, and Al^{3+}) were extracted using

177   0.1 mol L^{-1} BaCl$_2$, and their contents were measured using inductively coupled plasma

178   emission spectrometry (ICP-OES; USA). Activities of soil enzymes, including β-

179   glucosidase (BG) and cellulolytic enzyme cellobiohydrolase (CBH), were measured

180   using the method reported by Saiya-Cork et al. (2002). We added 1.0 g of soil sample

181   and 125 mL of 50 mmol L^{-1} acetate buffer solution to a triangular flask. The mixture

182   was homogenized for 5 min using a magnetic stirrer, and the supernatant was collected

183   as the test solution. In a 96-well microplate, the test solutions were applied to different

184   substrates (4-methylumbelliferyl-D-glucopyranoside and 4-methylumbelliferyl-D-

185   cellobioside) to measure BG and CBH activity, respectively. A multi-purpose

186   microplate reader (SpectraMax M5, USA) equipped with 365 nm excitation and 450

187   nm emission filters was used to measure fluorescence.

188

189   **2.6 Aboveground litter biomass and fine root biomass**

190   In each plot, three litter traps (1 m × 1 m) were installed approximately 1 m above

191   the ground surface. Around the 20th of each month, litter was collected from the

192   *Castanopsis fabri* forest. Litter from the same plot (with three traps) was collected and

193   mixed in the laboratory. The collected litter was dried and weighed to determine the

194   biomass. This process was continuously performed for one year. In December 2021, a

195   five-point sampling technique was used to collect fresh soil cores from each plot at a

196   depth of 0−10 cm, using a 3.5 cm-diameter soil auger. The diameter < 2 mm fine roots

197   in the soil cores were carefully selected and thoroughly washed, then dried at a constant

198   temperature of 60 °C, and finally weighed.

199

200   **2.7 Statistical analyses**

201   R 4.2.3 (R Core Team, 2023) and SPSS 27.0 (IBM, USA) were used to conduct

202   statistical analyses. One-way analysis of variance was examined to test the effects of N

203   addition on SOC fractions, soil physicochemical characteristics, exchangeable cations,

204   soil enzyme activities, aboveground litter biomass, and fine root biomass. The least

205   significant difference method was employed to compare treatment differences at a

206   significance level of $P < 0.05$. Principal component analysis was examined using the

207   "*factoextra*" package in R 4.2.3 to explore the influence of soil factors (exchangeable

208   cations contents), plant factors (aboveground litter biomass and fine root biomass), and

209   soil microbial factors (enzyme activities) on SOC fractions. Graphs were created using

210   Origin 9.1 software (OriginLab, USA).

211

212   **3 Results**

213   **3.1 Responses of SOC fractions to short-term N addition**

214   The POC content of soil samples was significantly affected by short-term N

215   addition in a content-dependent manner ($P < 0.01$), but N addition did not exert any

216   significant effect on the MAOC content ($P > 0.05$; Fig. 1a and 1b). Moreover, the

217   MAOC: POC ratio was significantly affected by N addition ($P < 0.01$), declining with

218   N addition (Fig. 1c).

219

220   **3.2 Responses of soil exchangeable cations and enzyme activities to short-term N**

221   **addition**

222   N addition had a significant impact on exchangeable cation content, except for Na^+

223   content. Specifically, the Al^{3+} content of the low-N addition and high-N addition groups

224   was significantly higher than that of control group, whereas the K^+, Ca^{2+}, and Mg^{2+}

225   contents were significantly lower than those of the control group ($P < 0.01$; Fig. 2).

226   With rising N-addition levels, soil enzyme activities (BG and CBH) reduced ($P < 0.01$;

227   Fig. 3).

228

229   **3.3 Responses of aboveground litter biomass and fine root biomass to short-term**

230   **N addition**

231   N addition significantly affected the aboveground litter biomass ($P < 0.01$). In

232   comparison to that in the control plots, the aboveground litter biomass in the low-N

233   addition and high-N addition plots was significantly risen by 17% and 28%,

234   respectively. In contrast, the fine root biomass was unaffected by the addition of N (P

235    $= 0.33$; Fig. 4).

236

237   **3.4 Analysis of key factors influencing SOC fractions changes**

238   Principal component analysis revealed a significant positive correlation between

239   POC and aboveground litter biomass ($R^2 = 0.48$), and significant negative correlations

240   between POC and soil exchangeable cations (Mg^{2+}, Ca^{2+}, K^+), pH, and soil enzyme

241   activities (BG, CBH) ($R^2_{POC-Mg} = 0.73$, $R^2_{POC-Ca} = 0.52$, $R^2_{POC-K} = 0.57$, $R^2_{POC-pH} = 0.82$,

242    $R^2_{POC-BG} = 0.88$, $R^2_{POC-CBH} = 0.91$). Moreover, we found that a close negative connection

243   between Al^{3+} and Ca^{2+} or K^+ ($R^2_{Al-Ca} = 0.57$, $R^2_{Al-K} = 0.55$; Fig. 5).

244

4 Discussion

4.1 Positive response of POC to short-term N addition

Our results demonstrated that short-term low and high N additions increased POC content ($P < 0.01$; Fig. 1a). This is in line with previous findings (Riggs et al., 2015; Chen et al., 2018; Chen et al., 2020) and confirms our first hypothesis. The positive response of POC to N addition can be attributed to the following reasons (Fig. 6). First, POC is predominantly formed by plant-based compounds (Cotrufo and Lavallee, 2022), so the rise in POC content following the addition of N could be due to the increase in the inputs of plant residues to soils. In our study, N addition enhanced aboveground litter biomass considerably, but not fine root biomass. This result in combination with the significant correlation between POC and aboveground litter biomass ($R^2 = 0.48$, Fig. 5) suggested that N-induced increase in POC was mainly derived from plant aboveground rather than belowground roots. Some previous studies suggested that POC pool is mainly affected by plant belowground roots (Zhang et al., 2019; Sokol et al., 2019; Sun et al., 2023). However, this phenomenon may be not appliable to our study, as N addition can enhance soil nutrient resources and decrease the proportion of biomass allocation to belowground and thus the amount of belowground C inputs (Feng et al., 2023). Second, the increase in POC may be also associated with the lower soil enzyme activities under N additions, as soil enzymes are important drivers of SOC decomposition (Liang et al., 2017). We found the significant decrease in soil enzyme activity in the low-N addition and high-N addition plots ($P < 0.01$; Fig. 3), and a significant negative relationship between soil enzyme activity and POC ($R^2_{POC-BG} = 0.88$, $R^2_{POC-CBH} = 0.91$; Fig. 4). This may be due to the leaching of soil exchangeable cations, which decrease soil pH, thereby inhibiting soil microbial biomass and soil enzyme activities (Averill and Waring, 2018), and reducing the decomposition and loss of POC. Overall, the increases in POC content under N addition can be not only related to N-induced increase in plant-derived C inputs but also related to N-induced decrease in microbial decomposition activities.

4.2 Non-significant response of MAOC to short-term N addition

N addition had a neutral effect on the MAOC content ($P = 0.27$; Fig. 1b), such result is contrary to our second hypothesis and previous studies (Lu et al., 2021; Rocci et al., 2021). Chen et al. (2021) showed that microbial turnover to produce microbial necromass is thought to be a major driver of MAOC, and the undetectable change in MAOC content might be regulated by maintaining an equilibrium between microbial necromass input and microbial decomposition output. Here, we attributed the non-response of MAOC to N addition to the following reasons. First, previous studies have shown that compared to aboveground litter, belowground roots make a greater contribution to the persistence of SOC, as they can interact with soil minerals, microorganisms, and aggregates right away after death (Wu et al., 2018; Whalen et al., 2021). However, fine root biomass was not influenced by N short-term addition, which may be a reason for the lack of a marked response of MAOC to N addition in this study. Second, the effect of N-induced soil acidification on adsorption and desorption of exchangeable cations, such as Ca^{2+}, Mg^{2+}, Fe^{3+}, and Al^{3+}, is also likely to be important factors regulating MAOC changes (Sun et al., 2023; Tang et al., 2023). However, we identified that few studies have reported the relationship between SOC pools and exchangeable cations following N addition (Yu et al., 2017; Chen et al., 2020; Sun et al., 2023). Specifically, Yu et al. (2017) demonstrated that fertilization promoted the enrichment of aluminium and iron ions on the surface of soil particles, leading to enhanced soil aggregation. This increased soil aggregation reduced the rate of soil mineralization, potentially increasing MAOC storage and preservation (Waychunas et al., 2005). In contrast, Chen et al. (2020) have indicated that N addition had no discernible effect on soil Al^{3+} content but decreased soil Ca^{2+} content (Chen et al., 2020; Sun et al., 2023). The reduction in Ca^{2+} content could potentially lead to decreased mineral sorption from microbial necromass and products, resulting in the depletion of MAOC content. In this study, a significant rise in soil Al^{3+} content and a simultaneous

301   decline in Mg^{2+}, Ca^{2+}, and K^+ contents were observed following the addition of N ($P <$

302   0.01; Fig. 2). Principal component analysis revealed a strong negative correlation

303   between Al^{3+} and Ca^{2+} (or K^+) ($R^2_{Al-Ca} = 0.57$, $R^2_{Al-K} = 0.55$; Fig. 5). In light of these

304   results, we suggest a new framework that the negative effects of leaching of

305   exchangeable cations on SOC bridging and the positive effects of exchangeable cation

306   dissolution on SOC adsorption may offset each other, leading to an insignificant

307   response of MAOC to the addition of N in this subtropical forest. Moreover, we

308   observed a decrease in MBC content under N addition (Table S1). Long-term changes

309   in microbial biomass may markedly affect MAOC by altering the adsorption of soil

310   minerals on microbial necromass or litters (Feng et al., 2022).

311

312   **4.3 Effects of short-term N addition on SOC persistence**

313   MAOC with a long turnover rate is critical for maintaining the persistence of SOC

314   (Kristensen et al., 2022). Although POC constitutes a smaller proportion of soil, its

315   faster turnover rate is essential for maintaining or improving soil fertility (Benbi et al.,

316   2011). Compared with MAOC, POC has a lower density, contains more chemically

317   complicated compounds on average, and has a higher C: N ratio (Cotrufo and Lavallee,

318   2022). Here, the MAOC: POC ratio was significantly decreased with the addition of N

319   ($P < 0.01$; Fig. 1c), indicating that N addition reduces the persistence and increases the

320   nutrient density of SOC (Sokol et al., 2022). Similar findings have been published by

321   Tang et al. (2023). There was a strong positive relationship between POC and SOC by

322   principal component analysis ($R^2 = 0.89$; Fig 5), suggesting that the change in SOC with

323   the addition of N was driven by a change in POC rather than MAOC. However, Lugato

324   et al. (2021) reported that POC in forest soils is susceptible to depletion under global

325   change. They recommended the implementation of coniferous forest management

326   strategies that are aimed at increasing plant inputs into the soil to counteract POC losses.

327   Overall, it is likely that the accumulation in POC under N addition will offset the loss

328   of POC with global change. A positive relationship between SOC loss and SOC

329   persistence was shown in Liang et al. (2023), suggesting that efforts should be directed

330   towards improving SOC persistence. Thus, we propose enhancing SOC persistence by

331   regulating soil exchangeable cations to facilitate mineral sorption of microbial

332   necromass and its products.

333

334   **5 Conclusion**

335   In this study, we elucidated that MAOC was less vulnerable to short-term N

336   addition than POC in a subtropical forest. The reason for the accumulation of POC

337   caused by N addition could be the combination of increased plant litter input and

338   decreased microbial decomposition. By contrast, short-term N addition did not exhibit

339   marked effect on MAOC. Such results could be related to cancellation of effects by the

340   lack of marked changes in fine root biomass and the adsorption and desorption of

341   organic molecules on the mineral surface. The marked decrease in MAOC: POC ratio

342   under N addition indicated that SOC persistence and nutrient density decreased. Overall,

343   our study links the responses of POC and MAOC to the plant–microbial–soil response,

344   providing insights into the mechanisms of SOC dynamics under ongoing N deposition

345   in subtropical forest ecosystems.

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347

348   **Data availability statement**

349   The data that supports the findings of this study are available in the supplementary

350   material of this article.

351

352

353   **Authors' contributions**

354   Qiufang Zhang, Yuehmin Chen and Jingqi Chen conceived and designed the study; Hui

355   Dai, Quanxin Zeng, Xueqi Sun, and Wenwei Chen collected the samples; Jingqi Chen

356   and Yuanzhen Peng analyzed the samples/data and wrote the manuscript with help from

357   Qiufang Zhang; Qiufang Zhang, Jiguang Feng, Biao Zhu, and Yuehmin Chen revised

358 the manuscript; all authors approved the final manuscript.

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360

361 **Competing interests**

362 The authors declare that they have no conflict of interest.

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553 **Figure captions**

554

555 **Fig. 1** Effects of N addition on the contents of particulate organic carbon (POC, **a**) and

556 mineral-associated organic carbon (MAOC, **b**) and MAOC: POC ratio (**c**). Boxes and

557 horizontal lines represent the quartile range and median, respectively, with lines

558 extending 1.5 times above and below the quartile range ($n = 4$). P represents the N

559 addition effect. Significant differences between various N addition treatments are

560 shown by different letters above the boxes ($P < 0.05$). CT, LN and HN represent control,

561 low-nitrogen and high-nitrogen, respectively, and the corresponding N addition rates

562 are 0, 40 and 80 kg N ha^{-1} yr^{-1}.

563

564

565 **Fig. 2** Effects of N addition on the content of exchangeable cations (K$^+$, **a**; Na$^+$, **b**;

566 Ca^{2+}, **c**; Mg^{2+}, **d**; Al^{3+}, **e**) in soil samples. Boxes and horizontal lines represent the

567 quartile range and median, respectively, with lines extending 1.5 times above and below

568 the quartile range ($n = 4$). P represents the N addition effect. Significant differences

569 between various N addition treatments are shown by different letters above the boxes

570 ($P < 0.05$). CT, LN and HN represent control, low-nitrogen and high-nitrogen,

571 respectively, and the corresponding N addition rates are 0, 40 and 80 kg N ha^{-1} yr^{-1}.

572

573

574 **Fig. 3** Effects of N addition on the activities of β-glucosidase (BG, **a**) and 1, 4-β-

575 cellobiohydrolase (CBH, **b**). Boxes and center lines represent the quartile range and

576 median, respectively, with lines extending 1.5 times above and below the quartile range

577 ($n = 4$). P represents the N addition effect. Significant differences between various N

578 addition treatments are shown by different letters above the boxes ($P < 0.05$). CT, LN

579 and HN represent control, low-nitrogen and high-nitrogen, respectively, and the

580 corresponding N addition rates are 0, 40 and 80 kg N ha^{-1} yr^{-1}.

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583 **Fig. 4** Effects of N addition on aboveground litter biomass (**a**) and fine root

584 biomass(**b**). Boxes and center lines represent the quartile range and median,

585 respectively, with lines extending 1.5 times above and below the quartile range ($n = 4$).

586   *P* represents the N addition effect. Significant differences between various N addition

587   treatments are shown by different letters above the boxes ($P < 0.05$). CT, LN and HN

588   represent control, low-nitrogen and high-nitrogen, respectively, and the corresponding

589   N addition rates are 0, 40 and 80 kg N ha^{-1} yr^{-1}.

590

591

592   **Fig. 5** Principal component analysis of soil organic carbon fractions and other soil

593   parameters. POC: particulate organic carbon; MAOC: mineral-associated organic

594   carbon; SOC: soil organic carbon; MN: mineral nitrogen; Litter: aboveground litter

595   biomass; Root: fine root biomass; BG: β-glucosidase; CBH: 1, 4-β-cellobiohydrolase.

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597

598   **Fig. 6** Conceptual diagram representing how the soil organic carbon (SOC) fractions

599   in the subtropical *Castanopsis fabri* forest react to the addition of N. Notably, N

600   addition increases particulate organic carbon (POC) content but exerts no marked

601   influence on mineral-associated organic carbon (MAOC) content; however, it reduces

602   SOC persistence. The white up, down, and horizontal arrows represent the positive,

603   negative, and neutral effects of N addition, respectively.

604   Note: ① N addition increases aboveground litter input, which enhances POC content.

605   ② N addition leads to soil acidification and inhibits soil enzyme activities. This

606   function potentially reduces POC decomposition, ultimately increasing POC content.

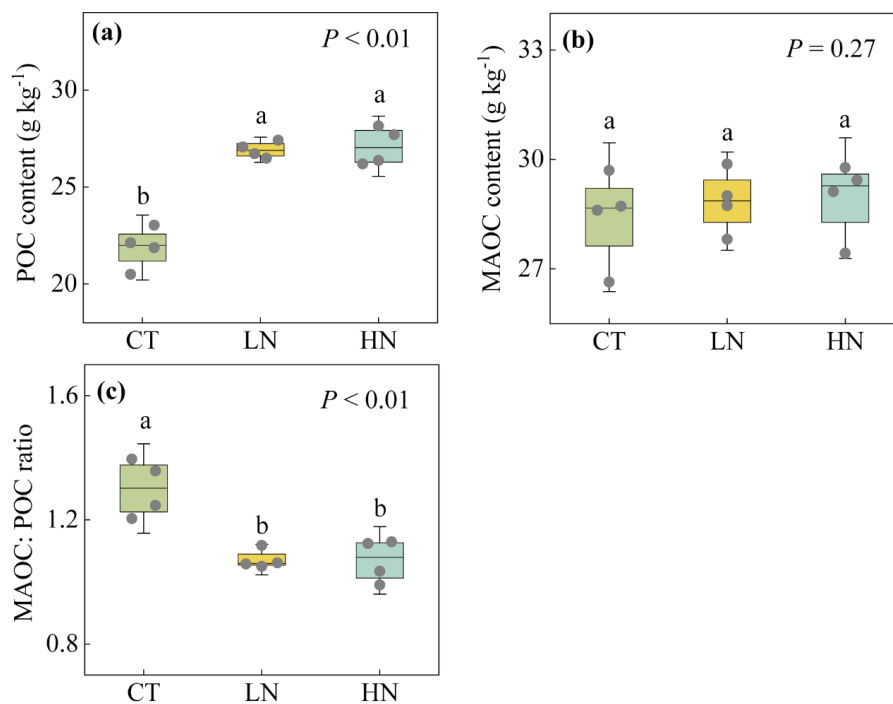
607   ③ Ca^{2+} and K$^+$ leaching negatively affect SOC bridging, whereas Al^{3+} dissolution

608   positively influences SOC adsorption. These opposing effects may offset each other,

609   resulting in a scenario in which MAOC content experiences no marked changes upon

610   N addition. ④ Fine root biomass remains largely unaffected by N addition,

611   contributing to the protection of soil aggregates and, therefore, to the preservation of

612   MAOC content.

613
614 **Fig. 1**
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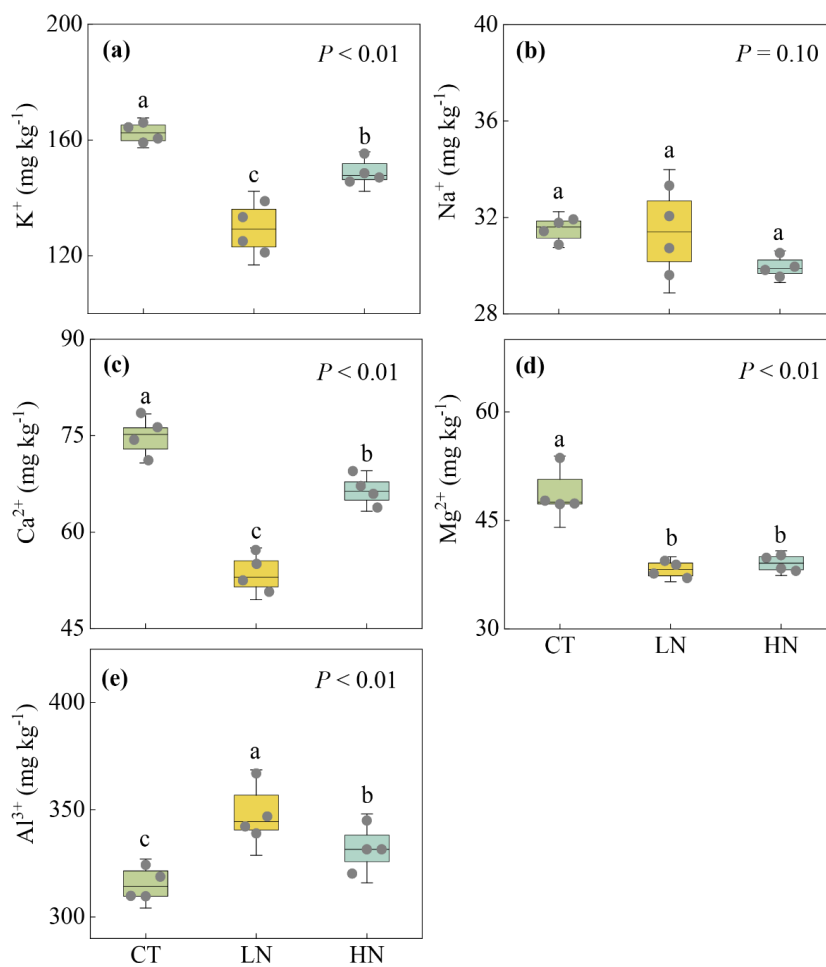
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617   **Fig. 2**

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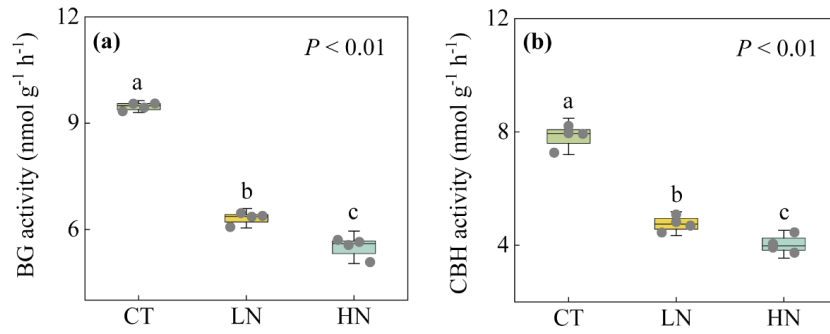
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620 **Fig. 3**

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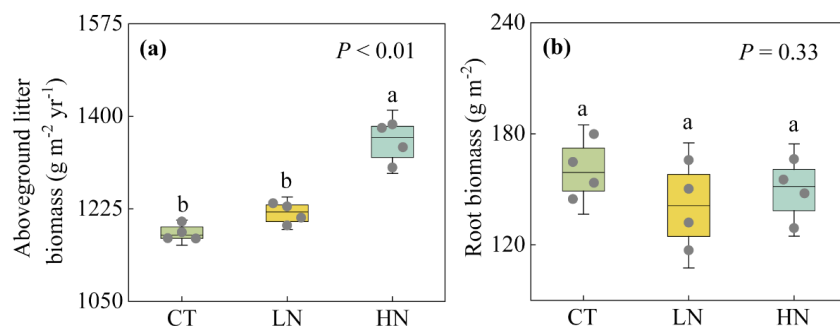
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623 **Fig. 4**

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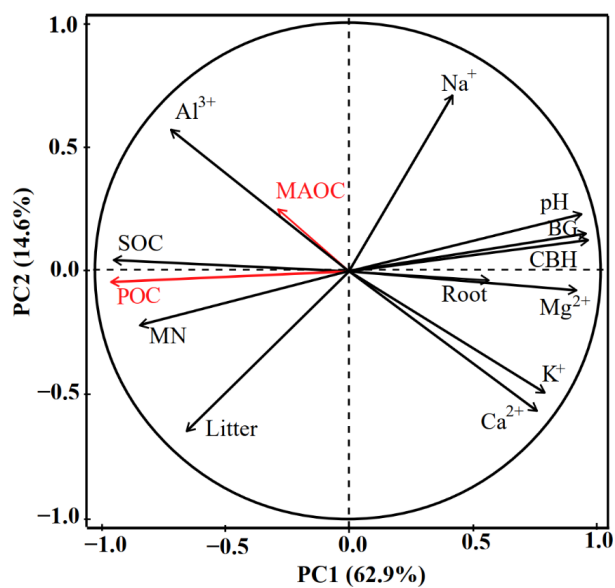
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626   **Fig. 5**

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629   **Fig. 6**

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