

**1Quantification of the sources of soluble organic N (SON) from new litter or
2indigenous soil in a typical subtropical forest**

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18Abstract

19 Decomposition of forest litter plays a major role in nitrogen (N) dynamics in soil. But to
20which extent that forest litter affects soil N and how much soil N is derived from the new
21litter under natural conditions remains unknown. An in-situ soil column experiment with 14-
22month litter decomposition was conducted to examine the effect of litter retention on soil N
23dynamics in a typical forest of subtropical China in 2018. Litter removal in the soil column
24was used as a control treatment, while natural litter or identical amount of ^{15}N labeled litter
25was added to soil columns as litter retention treatment. The results showed that litter removal
26caused a continuous decrease in concentration of soil soluble organic nitrogen (SON) in the
27first 5 months, and then SON began to accumulate and its concentration went up in spring
28showing obvious seasonal change. Litter retention accelerated the reduction of soil SON
29concentration in the first 2 months, while maintained a high concentration after that period.
30Soil $\text{NH}_4^+\text{-N}$ derived from litter was nitrified rapidly, and newly formed $\text{NO}_3^-\text{-N}$ was quickly
31immobilized or lost. Only 1.8% of soil SON came from litter N and 98.2% from indigenous
32soil N under the decomposition of labeled litter. Litter provided supplementation N to form
33new soil SON continuously, however, only a small part of SON was relatively stable, and
34SON played the role of reserve and regulatory pool. Soil SON and TN were formed after
35long-term litter accumulation and decomposition.

36

37*Keywords:* Soluble organic nitrogen (SON); ^{15}N abundance of soil N; ^{15}N labelled litter;

38Litter decomposition; N dynamics

391. **Introduction**

40 Soluble organic nitrogen (SON) is defined as the organic nitrogen (N) in the soil that can be
41 extracted by water or salt solution (Murphy et al., 2000; Zhong and Makeshin, 2003).

42 Previous studies have shown that soil SON has important ecological and environmental
43 functions (McDowell, 2003; Jones et al., 2004; Van Kessel et al., 2009; Liang et al., 2015),
44 and many of its unknown roles including characteristics, sources, sinks, and migration have
45 been revealed (Kalbitz et al., 2000; McDowell, 2003; Jones et al., 2004; Christou et al.,
46 2006; Kaiser and Kalbitz, 2012; Bu et al., 2014; Mauro et al., 2017; Ye et al., 2020). Its
47 existence in soil solution can provide an important and direct source of N to plants and soil
48 microorganisms (Murphy et al., 2000; Jones et al., 2004; Van Kessel et al., 2009; Rasmussen
49 and Kuzyakov, 2009; Moran-Zuloaga et al., 2015), and its leaching is an important process
50 of N losses (Fang et al., 2009; Van Kessel et al., 2009; Khalili and Nourbakhsh, 2012;
51 Camino-Serrano et al., 2014; Roth et al., 2019). SON reflects the physical, chemical, and
52 biological processes of the soil and has a profound impact on it (Zsolnay, 2003; McDowell,
53 2003, Christou et al., 2006; Klaus and Kalbitz, 2012). However, few field experiments have
54 been conducted to clarify the relative proportion of soil SON derived from exogenous N
55 (especially litter N) and the related formation mechanism (Neff et al., 2003; Quan et al.,
56 2018).

57 Approximately 90-95% of soil N exists in the form of organic N (Schulten and Schnitzer,
58 1998; Chen and Xu, 2008; Näsholm et al., 2009; Schmidt et al., 2014;). Plant litter is of
59 particular importance for soil N in forest (Zeller et al., 2000; Parton et al., 2007; Meiwes et
60 al., 2009; Eickenscheidt and Brumme, 2013; Huysen et al., 2013; Lehmann and Kleber,

612015; Wang et al., 2019), followed by roots, animal residues, microbe residues, atmospheric
62N deposition, and tree stemflow of N, etc. (Guo et al., 2013; Mauro et al., 2017; Cao et al.,
632019). After the inorganic N and some organic N with low molecular weight (LMW-SON) in
64the soil are assimilated by plants (Lipson and Näsholm, 2001; Karagatzides et al., 2009;
65Klaus and Kalbitz, 2012; Moran-Zuloaga et al., 2015), the N returned to soil is mainly in the
66form of forest litter. New litter is easy to decompose at the initial stage, and most of the litter
67N is quickly utilized by microorganisms and immobilized into the soil (Zeller et al., 2001;
68Guo et al., 2013; Roth et al., 2019). The remaining litter that is difficult to decompose exists
69in the form of residue, and the residue enters the soil after being broken and decomposed
70(Hatton et al., 2012; Klaus and Kalbitz, 2012; Eickenscheidt and Brumme, 2013; Lehmann
71and Kleber, 2015). Previous studies have shown that forest litter could provide an abundant
72carbon source which increased the N demand by microorganisms in the form of $\text{NH}_4^+\text{-N}$
73while reduced $\text{NO}_3^-\text{-N}$ loss, resulting in an increase in soil N retention capacity (Blumfield et
74al., 2004; Cheng et al., 2014; Quan et al., 2018). However, study has also shown that litter
75would increase N availability which could increase the abundance of ammonia-oxidizing
76archaea and bacteria (AOA and AOB, respectively), and thus accelerate $\text{NO}_3^-\text{-N}$ production
77and resulting in a higher rate of denitrification (Wieder et al., 2013). A part of organic N
78released by new litter can directly enter the soil, and it will form a part of SON with fast
79turnover (Kalbitz et al., 2000; Kiikkilä et al., 2006; Hatton et al., 2012). The effect of litter
80on soil SON cannot be ignored, though little SON derived from forest litter reach the mineral
81subsoil (Zeller et al., 2000; Klaus and Kalbitz, 2012). Thus, to which extent that forest litter
82affects soil N (especially SON) and how much soil N is derived from the new litter under

83natural conditions remain to be explored.

84 The SON in soil can be divided into two parts including labile part with fast turnover and
85the recalcitrant part that is relatively stable, and previous studies have concluded that SON is
86dominated by recalcitrant part (Kaiser and Kalbitz, 2012; Quan et al., 2018; Roth et al.,
872019.). The abundance of SON in the soil will change significantly with the decomposition
88of forest litter, and the characteristics of newly formed soil SON derived from litter are
89needed to be explored. Isotopic pool dilution using ^{15}N employed has been proved to be a
90valuable tool for increasing our understanding of N cycling processes (Zeller et al., 2000;
91Murphy et al., 2003; Templer et al., 2007; Cheng et al., 2014; Wang et al., 2015). Soil
92microorganisms play a very complicated role in this process (Neff et al., 2003; Wieder et al.,
932013; Chen et al., 2018; Quan et al., 2018; Roth et al., 2019; Ye et al., 2020), since it is
94difficult to estimate how much N derived from soil microorganisms through the assimilation
95of litter. In this study, we focused on that how much litter N can enter the soil to form new
96soil N, and paid seldom attention to how much N being transformed by microorganisms in
97soil.

98 Subtropical forests are globally important in providing ecological goods and services (Ali
99et al., 2017). During the past few decades, climatic climax vegetation has largely converted
100into evergreen broadleaved forest in the subtropical region of China, and the restoration of
101evergreen broadleaved forest reflects a natural succession process (Zeng et al., 2014; Xiang
102et al., 2016; Chen et al., 2018). Subtropical forests represent 11% of world forests, and
103occupy approximately 320.1 million hectares, with approximately 55.0 million hectares in
104the China (FAOUN, 2020). Soil N cycling in subtropical forest ecosystems has received a

105widespread attention, especially with the change in climate and the continuous increase of
106atmospheric N deposition. Studies on the effects of forest litter on soil SON within
107subtropical forests and regional N cycling in China is warranted. Hence, we conducted a
108field experiment to track the dynamics of ^{15}N abundance and ^{15}N mass of soil N pools by
109adding ^{15}N -labeled forest litters to in-situ undisturbed soil columns set in a typical
110subtropical forest. We hypothesized that 1) litter retention plays an important role in soil N
111dynamics; 2) The ^{15}N abundance and ^{15}N mass of soil SON would gradually increase during
112the decomposition of forest litter; and 3) the relative proportion of soil SON derived from
113litter would be less but gradually increase. This study aimed to provide an in-depth
114understanding of the behavior and source of soil SON under natural conditions and its
115potential drivers in subtropical forests.

116

117**2. Materials and methods**

118*2.1. Site description*

119 This study was carried out in Dashanchong Forest Park (N28°23'58"–28°24'58",
120E113°17'46"–113°19'08") in Changsha, Hunan Province, China (Fig. 1). The park has a
121typical humid mid-subtropical monsoon climate, the mean annual precipitation ranges from
1221412 to 1559 mm, and the primarily precipitation occurs from April to August. The mean
123annual air temperature is 16.6 - 17.6°C, with the maximum and minimum temperatures
124being 39.8 and –10.3 °C, respectively. The soil type is a well-drained red soil developed
125from slate parent rock, and classified as an Alliti-Udic Ferrosol, corresponding to Acrisol
126according to the World Reference Base for Soil Resources (WRB). One naturally restored

127 forest in the park dominated by broad-leaved tree species was selected for this study (Zeng et
128 al., 2014; Chen et al., 2018). A plot of approximately 1 ha was established in this forest for
129 long-term observation, and stand characteristics and soil physical and chemical properties
130 are described in Table 1.

131

132 2.2. The field experiment design and implementation

133 2.2.1 Preparation of ^{15}N labeled litter and natural litter

134 In March 2017, 28.0 kg of red-yellow soil and 12.0 kg of river sand were fully mixed, and
135 then base fertilizers (NaH_2PO_4 , 0.3 g P_2O_5 kg^{-1} ; KCl , 0.3 g K_2O kg^{-1}) were applied in the
136 above mixture. The mixed soil was divided into 2 pots, 18 plants of *Quercus glauca*
137 seedlings were planted per pot for 1 year. Nitrogen fertilizer was applied to the plant roots
138 using 30 atom% ^{15}N labeled ammonium sulfate solution in every 1-2 months, the total
139 applied amount of ammonium sulfate was approximately 126.5 g. The plants were harvested
140 after 1-year growth and used as labeled litter, and then the N content and ^{15}N abundance in
141 the plants were determined (Table 2).

142 At the same time, four litter collection traps were set up on the study site to collect natural
143 litter. The trap was a circle with an area of 1 m^2 , and a conical collection bag was made in the
144 middle with 40 mesh nylon net. Litters were collected regularly every 2 months for one year,
145 brought back to the laboratory, dried at constant temperature of 80 $^\circ\text{C}$, and weighed after
146 completely dry.

147 2.2.2 The field experiment and soil sampling

148 In August 2018, 57 steel pipes with a diameter of 8 cm and a depth of 25 cm were
149 randomly inserted into the study plot, and each soil column was about 30 cm apart. The top

150 of steel pipe was 5 cm above the ground to form a soil column, and the surface litter in
151 column was removed carefully. Three soil columns were dug out directly and brought back
152 to the laboratory to determine the initial soil N concentrations. The remaining 54 soil
153 columns were divided into three groups, and 20-mesh nylon meshes were added on top of
154 the pipes. One group of soil columns was added natural litter at 5 g per column (about two-
155 years of fallen litter), and another group was added labeled litter at 5 g per column (^{15}N -
156 6.69 atom%). The remaining soil columns were used as the control group (litter removal
157 treatment). According to our previous studies, the turnover rate of surface litter in this plot
158 was also approximately two years, thus 5 g of litter per column was reasonable to represent
159 all recent litters fallen on the ground. The properties of litters are shown in Table 2.

160 For each group (or treatment), 3 soil columns were collected in every 2-3 months, and for
161 a total of 6 times (i.e. September 31 of 2018 and January 1, April 1, June 1, August 2, and
162 September 30 of 2019). After soil columns were brought back to the laboratory in each time,
163 all the decomposed litters were taken out firstly, and then the soil samples were collected and
164 separated into three layers. The collection order of the three layers of soil was: 0-5 cm from
165 top, 10-20 cm at the bottom, and 5-10 cm in the middle. The litters were washed with
166 ultrapure water, and dried at 80 °C in a constant-temperature drying oven and weighed after
167 completely dry. Fresh soil samples were fully mixed after roots and visible stones were
168 picked out, and were sieved through a 20-mesh sieve and weighed immediately. Soil samples
169 were stored in a cold room at 4°C prior to use. A portion of every sieved soil sample was air-
170 dried, ground to pass through a 100-mesh sieve for analysis of total nitrogen (TN).

171

1722.3. *Chemical analysis*

173 The fresh soils were extracted with 2 mol L⁻¹ KCl solution with a soil: solution ratio of 1:5
174(w/v). Each soil and solution mixture was shaken at a 180-rpm constant temperature shaker
175for 0.5 h at 25 °C and then passed through a 0.45- μ m filter. The soluble N in the soils was
176measured using the Kjeldahl method. 20 mL of the extract was steam-distilled with MgO to
177separate out the NH₄⁺-N and was distilled again after the addition of Devarda's alloy to
178separate the NO₃⁻-N on a steam distillation system (Bremner, 1996). The NH₃ was distilled
179out separately and was trapped in 50-mL polyethylene plastic bottles using the 20 g L⁻¹ boric
180acid solution. Total soluble nitrogen (TSN) in the extract was determined by semi-micro
181Kjeldahl digestion using TiO₂, CuSO₄ and K₂SO₄ as catalysts. The digested extract was
182transferred to the steam distillation system, and NH₃ was distilled out with 10 mol L⁻¹ NaOH
183solution. The SON was calculated as the difference between TSN and soluble inorganic N
184(NH₄⁺-N and NO₃⁻-N).

185 The TSN, NH₄⁺-N, and NO₃⁻-N (had been converted to NH₄HB₄O₇·3H₂O and/or
186(NH₄)₂B₄O₇·4H₂O solutions) were separated for ¹⁵N measurements by distillation with 10
187mol L⁻¹ NaOH solution again. The NH₃ was distilled out separately and was acidified and
188converted to (NH₄)₂SO₄ using 0.005 mol L⁻¹ H₂SO₄ solution (Cheng et al., 2018). The
189(NH₄)₂SO₄ solutions were packed in 50 mL polyethylene plastic bottles with a small round
190piece of glass fiber membrane (GF/D Whatman 1823-047) being placed. Solutions with a
191glass fiber membrane were evaporated to dryness at 80 °C in a constant-temperature
192drying oven, and were analyzed for ¹⁵N abundance. Soil moisture content was measured by
193drying sub-samples of the soil at 105°C to a constant weight. The total organic carbon (TOC)

194in the soil and litters were measured using the Walkley-Black $K_2Cr_2O_7-H_2SO_4$ digestion
195method. The TN in the soil and litters were measured using the Kjeldahl method. The
196digested extract of soil TN was also transferred to the steam distillation system for
197distillation and separated for ^{15}N measurement as above.

198.

1992.4 Calculations and statistical analysis

200 ^{15}N abundance (atom%) in a glass fiber membrane containing solid $(NH_4)_2SO_4$ was
201determined using an isotope mass spectrometer (MAT-251, USA) and calculated as follows
202(Quan et al., 2018):

$$203 \quad SO^{15}N \text{ abundance (atom\%)} = \{ [TS^{15}N \text{ atom\%} \times TSN \text{ concentration (mg kg}^{-1})] - [^{15}NH_4^+ \text{-N atom\%} \times NH_4^+ \text{-N concentration} \\ 204 \quad \text{(mg kg}^{-1})] - [^{15}NO_3^- \text{-N atom\%} \times NO_3^- \text{-N concentration (mg kg}^{-1})] \} / SON \text{ concentration (mg kg}^{-1}) \quad (1)$$

$$205 \quad ^{15}N \text{ mass (mg)} = ^{15}N \text{ abundance (atom\%)} \times N \text{ concentration (mg kg}^{-1}) \times \text{weight of fresh soil in each layer (kg)} \times [1 - \text{soil} \\ 206 \quad \text{moisture content (\%)}] \quad (2)$$

$$207 \quad ^{15}N \text{ recovery (\%)} = \text{Increased } ^{15}N \text{ mass in soil column (mg)} \times 100\% / \{ [\text{Initial labeled litter mass (g)} - \text{residual litter} \\ 208 \quad \text{mass (g)}] \times \text{litter TN concentration (g kg}^{-1}) \times ^{15}N \text{ abundance (atom\%)} \text{ of labeled litter} \} \quad (3)$$

$$209 \quad \text{The proportion of soil N derived from litter (\%)} = (^{15}N \text{ abundance of soil N} - ^{15}N \text{ abundance of background soil N}) / (^{15}N \\ 210 \quad \text{abundance of labeled litter} - ^{15}N \text{ abundance of background soil N}) \times 100\% \quad (4)$$

211 The concentrations of all soil samples were expressed on the basis of dry soil weight. We
212used one-way analysis of variance (ANOVA) and Tukey's honestly significant difference test
213to determine the effects of different litter treatments on soil N concentrations in each period,
214and significant changes in ^{15}N abundance, ^{15}N mass, and ^{15}N recoveries of soil N among
215different periods throughout the entire experimentation period. All statistical analyses were
216performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

217

2183. Results

2193.1. Soil N concentration in litter retention and removal treatments

220 The SON concentration in soil decreased during the first 5 months (till winter, January 1
221in 2019) in litter removal treatment, and then began to rise in spring and reached its peak on
222June 1 in the following year (Fig. 2a, Table s1). However, SON concentration decreased
223rapidly only in the first 2 months, and then increased gradually afterwards and reached its
224peak also around June 1 (in 10th month) in litter retention treatment.

225 The NH_4^+ -N concentrations in soil decreased rapidly in the first 2 months in all three
226treatments, and were higher in litter retention treatment than in litter removal treatment (Fig.
2272b). The NO_3^- -N concentration in soil continued to decrease in the first 8 months in litter
228removal treatment (Fig. 2c). However, NO_3^- -N concentrations were raised in the initial 2
229months in litter retention treatment, and then decreased rapidly later. After April 1, 2019 (8
230months later), NO_3^- -N concentration increased slowly in three treatments, and was always
231higher in litter removal treatment than in litter removal treatment. No significant difference
232in TN concentration was observed among the three treatments during the entire period
233($p>0.05$, Table s1), except a decrease in litter retention treatment in the initial 2 months (Fig.
2342d).

235

2363.2 ^{15}N abundance and ^{15}N mass of soil N under the decomposition of labeled litter

237 In general, with the decomposition of labeled litter, the ^{15}N abundance of SON in soil
238showed an overall upward trend, except a decrease in April 1, 2019 (in 8th month).

239However, the ^{15}N mass of SON decreased in the first 2 months, and then increased gradually.

240 The change trends of the ^{15}N abundance or ^{15}N mass of soil SON were basically identical in
241 the 0-5 cm and 5-10 cm layers. The change trends of the ^{15}N abundance and ^{15}N mass of
242 SON in the 10-20 cm layer were delayed compared with those of the upper two layers (Fig.
243 a).

244 The ^{15}N abundance of $\text{NH}_4^+\text{-N}$ in soil increased gradually within 10 months, and decreased
245 later (Fig. 3b). The ^{15}N mass of $\text{NH}_4^+\text{-N}$ decreased gradually over time, except an increase on
246 June 1, 2019 (in 10th month). The ^{15}N abundance and ^{15}N mass of $\text{NO}_3^-\text{-N}$ in soil were raised
247 rapidly in the first 2 months, and then decreased gradually after that period. After April 1 (in
248 8th month), ^{15}N abundance and ^{15}N mass of $\text{NO}_3^-\text{-N}$ increased gradually again. The ^{15}N
249 abundance and ^{15}N mass of TN in soil basically increased over time.

250

251 3.3 ^{15}N recoveries of soil N and the proportion of soil N derived from labeled litter

252 The ^{15}N recoveries of SON in soil were 0.27%~0.57% and increased gradually during the
253 whole experimentation period (Fig. 4a). ^{15}N recoveries of $\text{NH}_4^+\text{-N}$ were around 0.21%,
254 except for an increase up to 0.87% on June 1, 2019 (in 10th month). ^{15}N recoveries of $\text{NO}_3^-\text{-N}$
255 were decreased rapidly in first 8 months, and thereafter increased gradually. ^{15}N recoveries
256 of TSN and TN in soil decreased rapidly in first 8 months with the same trend as $^{15}\text{NO}_3^-\text{-N}$,
257 and then increased gradually after 8 months (Fig. 4b). The average ^{15}N recoveries in TSN
258 pool and TN were 4.5% and 34.0%, respectively.

259 The proportion of soil SON derived from litter increased rapidly from 0.36% to 4.6% in the
260 first 5 months, and then decreased later (Fig. 5a, Table s2). The average proportions of SON
261 derived from litter were 2.77%, 1.48% and 1.29% in 0-5 cm, 5-10 cm, and 10-20 cm layers,

262respectively. The proportion of soil NH_4^+ -N derived from litter increased gradually in the
263first 10 months and then decreased later (with an average of $1.75\% \pm 1.10\%$). The proportion
264of soil NO_3^- -N derived from litter increased rapidly in first 2 months, and then decreased till
2658 months, and increased gradually again after that. The proportion of soil TN derived from
266litter increased in the first 5 months, and remained stable after that, except a decrease on
267April 1 (in 8th month). The average proportions of soil TN derived from litter were 2.55%,
2680.79%, and 0.34% in 0-5 cm, 5-10 cm, and 10-20 cm soil layers, respectively.

269

2704. Discussion

2714.1 *Effects of litter on soil N concentration*

272 As forest litter is the main source of nutrient and energy input into the soil, it significantly
273affects soil activity and N cycling (Zeller et al., 2001; Ruan et al., 2005; Cusack et al., 2009;
274Shilenkova and Tiunov, 2013). Earlier results showed that a large amount of the N released
275from litter was retained as organic N in the soil, and release of litter N was correlated to litter
276mass loss (Zeller et al., 2000, 2001; Blumfield et al., 2004; Hatton et al., 2012). Wang et al.
277(2015) conducted a 9-month litter decomposition experiment using PVC cylinders to
278examine the effect of litter removal and addition on soil N dynamics in a subtropical forest;
279however, results showed that soil N transformations were not significantly affected by
280decomposing litter. An experiment conducted in a tropical forest using litter bag showed that
281soil dissolved N concentration was significantly correlated to litter quantity (Zhou et al.,
2822015). Previous studies have also shown litter removal could decrease nutrient supply for
283soil microorganisms and substantially suppress soil activities (Ruan et al., 2005; Chen et al.,
2842014).

285 In the field experiment of this study, the SON concentration continued to decrease in first
2865 months after litter was removed, and then began to rise in spring and reached its peak on
287June 1 in the following year (Fig. 2a). This indicated that litter removal caused the
288continuous mineralization and/or utilization of soil SON, and SON began to accumulate and
289its concentration went up in spring due to significant change in climatic conditions
290(increased temperature and moisture) (Huysen et al., 2013; Zhou et al., 2015; Wang et al.,
2912015). Compared with litter removal treatment, litter retention treatment accelerated the
292mineralization and/or utilization of soil SON in the first 2 months; this could be
293characterized by pronounced decrease in SON concentration in easily decomposable labeled
294litter (a low C/N ratio) addition treatment (Fig. 2a). This may be explained by the fact that
295litter decomposition significantly promoted soil activities (altered community structure and
296activities of soil biota, and increased activities of enzymes, etc.) and accelerated soil N
297transformation; and more SON was needed to satisfy mineralization and/or utilization by
298microorganisms (Ruan et al., 2005; Caner et al., 2004; Guo et al., 2013; Hobbie, 2015). After
2992 months, soil SON concentration rose rapidly in litter retention treatment and was higher
300than litter removal treatment, showing that litter could supplement the loss of soil SON in
301this period. The change trends of soil SON concentration in three soil layers were basically
302identical; the only difference was concentration in the surface layer being higher. Studies
303have shown that the low SON concentration in deep soil layer was due to the adsorption of
304SON by soil clay minerals in surface layer (Kalbitz et al., 2000), and SON in deep soil was
305mainly dominated by microbial residues (Klaus and Kalbitz, 2012; Roth et al., 2019). Thus,
306forest litter accelerated the mineralization and/or utilization of soil SON in the short term,

307 and maintained a high SON concentration in the long term.

308 In the litter removal treatment, NH_4^+ -N concentration in soil remained basically stable
309 after the initial decline (Fig. 2b). It showed that since there was no continuous supplement of
310 exogenous litter N and the intensity of nitrification was not enhanced; the mineralization of
311 soil organic N basically met with the consumption of NH_4^+ -N in the soil (Wieder et al.,
312 2013). However, NH_4^+ -N concentration decreased in litter retention treatment throughout the
313 entire period. This might be due to the increased NH_4^+ -N demand by soil microorganisms,
314 despite litter was continuously decomposed to replenish NH_4^+ -N (Cheng et al., 2014; Quan et
315 al., 2018).

316 After litter was removed, soil NO_3^- -N concentration continued to decrease within 8
317 months. NO_3^- -N was leached to the bottom soil layer or lost by continuous consumption in
318 the denitrification process or immobilized by microorganisms since there was no continuous
319 supplement of exogenous litter N and not enough soil NH_4^+ -N. This was consistent with the
320 finding reported by Wieder et al. (2013) that litter removal significantly reduced the rate of
321 gross soil N mineralization and decreased the rate of gross soil nitrification in a tropical
322 forest. However, NO_3^- -N concentration increased rapidly in litter retention treatment in the
323 first 2 months, showing that soil nitrification was significantly enhanced and soil NH_4^+ -N
324 derived from exogenous litter N was rapidly nitrified by microorganisms. The labeled litter
325 addition enhanced soil nitrification more significantly, and the time required for NO_3^- -N
326 concentration to reach the maximum may be less than 2 months (Fig. 2c). This was not found in
327 previous studies, and it reminded us that the dynamic changes of soil N were obvious in
328 initial stage of litter decomposition. After 2 months, NO_3^- -N concentration showed a rapid

329 downward trend till 8 months later (spring, April 1 in 2019), indicating that the soil $\text{NH}_4^+\text{-N}$
330 supply was insufficient and $\text{NO}_3^-\text{-N}$ was immobilized or lost during this period. The $\text{NO}_3^-\text{-N}$
331 concentration rose again after spring in 2019 in all three treatments, indicating that
332 nitrification process followed clear seasonal patterns, so the effect of seasonality on the soil
333 N needed to be considered (Wang et al., 2015). The change trends of soil $\text{NO}_3^-\text{-N}$
334 concentration in three soil layers were identical, and the rapid increase in soil $\text{NO}_3^-\text{-N}$
335 concentration in 5-10 cm and 10-20 cm layers in the first 2 months indicated that $\text{NO}_3^-\text{-N}$ had
336 a significant leaching loss (Eickenscheidt and Brumme, 2013; Cheng et al., 2014; Wieder et
337 al., 2015). Under the three treatments, TN concentration in the soil did not change
338 significantly during the entire period ($p>0.05$). There was a decrease in soil TN
339 concentration in litter retention treatment relative to the other treatments during the initial
340 period, showing that litter enhanced soil activity rapidly, and more N in the soil would be
341 lost under the action of denitrification or leaching of nitrate.

342

343 4.2 Changes in the ^{15}N abundance, ^{15}N mass, and ^{15}N recoveries of soil N under the 344 decomposition of labeled litter

345 The ^{15}N abundance of soil N reflects ^{15}N of N source and N isotope fractionations, which
346 could provide insights into the mechanisms and processes influencing soil N dynamics
347 during litter decomposition in forest ecosystems (Templer et al., 2007; Huysen et al., 2013;
348 Wang et al., 2015). Here, we also calculated the ^{15}N mass of soil N in the soil column, which
349 could help to better understand the dynamic changes of soil N derived from litter. Results
350 showed that the ^{15}N abundance of SON increased but the ^{15}N mass of SON decreased in the

351 first 2 to 5 months (Fig. 3). It indicated that ^{15}N labeled litter could continuously provide
352 supplementation of N to form new SON, but soil SON was mineralized into $\text{NH}_4^+\text{-N}$ and/or
353 assimilated by microorganisms at the same time; so only a small portion of SON derived
354 from litter could be stored in soil SON pools. The newly formed SON derived from litter
355 was not preferentially used by microorganisms; a part of it was accumulated. Thus, most of
356 soil SON derived from litter was labile and a small part was relatively stable (Kiikkilä et al.,
357 2006; De Troyer et al., 2011). Within a 14-month period, an overall upward trend in the ^{15}N
358 recovery of soil SON (Fig.4) showed that SON had accumulative characteristics under the
359 supplement of exogenous litter N. Therefore, it can be speculated that soil SON was a
360 relatively stable reserve and regulatory pool of soil N.

361 With the decomposition of labeled litter, the ^{15}N abundance of soil $\text{NH}_4^+\text{-N}$ showed an
362 overall upward trend (Fig.3), indicating that litter N could continue to be mineralized to
363 supplement soil $\text{NH}_4^+\text{-N}$. However, the ^{15}N mass of soil $\text{NH}_4^+\text{-N}$ showed an overall
364 downward trend, indicating that soil $\text{NH}_4^+\text{-N}$ was continuously being consumed. In the 10th
365 month (in summer, June 1, 2019), the ^{15}N abundance and ^{15}N mass of $\text{NH}_4^+\text{-N}$ suddenly
366 increased, which was due to increase in litter decomposition rate and N mineralization under
367 climatic conditions of higher temperature and humidity in summer (Eickenscheidt and
368 Brumme, 2013). The ^{15}N recovery of soil $\text{NH}_4^+\text{-N}$ did not change significantly except in the
369 10th month (Fig. 4). Both the ^{15}N abundance and ^{15}N mass of $\text{NO}_3^-\text{-N}$ increased significantly
370 in the first 2 months, once again, showing that $\text{NH}_4^+\text{-N}$ derived from labeled litter N was
371 rapidly nitrified. From the 2th month to 8th month, both the ^{15}N abundance and ^{15}N mass of
372 $\text{NO}_3^-\text{-N}$ showed a rapid decline, indicating that newly formed soil $\text{NO}_3^-\text{-N}$ could be quickly

373 immobilized or lost. After 8th month (in spring), the ^{15}N abundance and ^{15}N mass rose at the
374 same time, the released litter N slowly entered the soil NO_3^- -N pool again. The ^{15}N recovery
375 of soil NO_3^- -N showed the identical trend as the changes in ^{15}N abundance and ^{15}N mass
376 (Fig. 4a).

377 Compared with the slow rise of ^{15}N abundance of soil SON, the rapid rise of ^{15}N
378 abundance of NO_3^- -N in the first 2 months reflected that small portion of NO_3^- -N would be
379 immobilized in the SON, because the ^{15}N abundance of soil SON would rise rapidly as NO_3^- -
380 N if significant immobilization occurred (Quan, et al., 2018). The ^{15}N recovery of soil SON
381 and NH_4^+ -N were much lower than the ^{15}N recovery of NO_3^- -N, indicating that the
382 transformation of SON and NH_4^+ -N derived from litter N into the NO_3^- -N was very quick,
383 and NO_3^- -N was the final stored-product in soil. Throughout the entire period, the ^{15}N
384 abundance and ^{15}N mass of TN in soil showed an overall upward trend, indicating that litter
385 N would continue to enter the soil. The ^{15}N abundance and ^{15}N mass of soil TN in surface
386 layer were significantly higher than those of the bottom layer, showing exogenous litter N
387 was mainly concentrated in the surface layer. These findings were similar to previous studies
388 that the ^{15}N released from litter remained at the soil surface after 3 yr litter decomposition
389 (Zeller et al., 2000; 2001; Blumfield et al., 2004; Hatton et al., 2012). Overall,
390 decomposition of litter plays a major role in N dynamics in soil (Huysen et al., 2013;
391 Eickenscheidt and Brumme, 2013; Guo et al., 2013).

392

393 4.3 Proportion of soil N derived from labeled litter

394 The proportion (0.36%~4.65% during 14 months, Fig. 5a) of soil SON derived from litter

395 in this study was low than that from an incubation experiment in laboratory (Quan, et al.,
396 2018), showing that a lower proportion of SON derived from litter can be stored in the soil
397 and N turnover rate is much higher under field conditions. We calculated that approximately
398 98.2% of soil SON came from indigenous soil N and only 1.8% of soil SON came from litter
399 N. The proportion of soil NH_4^+ -N derived from litter (0.35%~3.99%, mean 1.8%) was
400 basically close to that of soil SON derived from litter, and was only a little less. This
401 indicated that the contributions of litter N to these two N components was both relatively
402 minor, and the conversion of SON was slightly slower than that of NH_4^+ -N.

403 The proportion of soil TN derived from litter (0.31%~3.65%, mean 1.2%) was slightly
404 less than those of the above two components, showing indigenous soil TN has a strong
405 buffering capacity. The proportion of litter N entering the soil was very small in a short time,
406 thus soil N was accumulated and formed after long-term litter decomposition. In this study,
407 litter N entering the soil under the decomposition of two-years litter during one year only
408 accounted for less than 2%, therefore, this indicated that it takes at least 100 years of litter to
409 form all new soil N. Coincidentally, Tipping et al. (2012) found that dissolved organic matter
410 becoming stable soil organic matter had mean residence times of 100-200 years. The
411 proportions of soil SON and TN derived from litter in 0-5 cm layer were significantly higher
412 than those of the bottom soil, thus soil N derived from litter was mainly concentrated in
413 surface soil. This was because of the fact that organic matters in the soil were more difficult
414 to reach to deep soil layer than inorganic matters (Chen and Xu, 2008; Hatton et al., 2012;
415 Klaus and Kalbitz, 2012). However, the proportion of soil NO_3^- -N derived from litter
416 (1.25~19.86%) was higher, approximately 10.0% of soil NO_3^- -N came from litter N. It

417 indicated that most of the mineralized litter N was converted into NO_3^- -N as the end product.
418 The peak of proportion of soil NO_3^- -N derived from litter N (in 2th month) was observed
419 much earlier than the peaks of proportions of soil SON (in 5th month) and soil NH_4^+ -N (in
420 10th month) derived from litter N (Fig. 5a), indicating that NH_4^+ -N derived from litter N was
421 preferentially utilized by microorganisms during the nitrification process in soil N cycling.
422 Many soil processes are driven or mediated by microorganisms, and microorganisms play
423 very complex roles in the soil N cycling (Neff et al., 2003; Wieder et al., 2013; Quan et al.,
424 2018; Roth et al., 2019). Not only microorganisms can use litter N directly, but they are both
425 the source and sink of soil soluble organic N at the same time. Therefore, in this study, we
426 did not pay attention to the changes in ^{15}N abundance of soil microbial N. Future works need
427 to focus on more complex N-related processes such as the roles of microorganisms and the
428 contribution of other exogenous N to soil N (Fig. 5b).

429

430 **5. Conclusions**

431 Litter retention accelerated the reduction of soil SON in the first 2 months, while
432 maintained a high SON concentration thereafter. Litter continuously supplemented N for
433 forming new soil SON, however, only a small portion of SON derived from litter was
434 relatively stable, and SON played the role of reserve and regulatory pool of soil N. Soil
435 NH_4^+ -N derived from litter was nitrified rapidly; newly formed NO_3^- -N was quickly
436 immobilized or lost. The TN concentration in the soil did not change significantly during the
437 entire period, except a decrease in the first 2 months in litter retention treatment relative to
438 litter removal treatment. The above results could be explained by the fact that litter

439decomposition increased nutrient supply to soil microorganisms and thus enhanced soil
440activities. The proportions of soil SON and TN derived from litter were significantly higher
441in 0-5 cm layer than the bottom soil, indicating that litter N mainly concentrated in the
442surface layer. Only 1.8% of soil SON came from litter N; approximately 98.2% of soil SON
443came from indigenous soil N under the decomposition of two-years of fallen litter during one
444year. The same proportion was observed in soil NH_4^+ -N. Thus, the proportion of litter N
445entering the soil was small in a short time, and soil SON and TN were formed after long-
446term litter accumulation and decomposition in a subtropical forest.

447

448

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453

454**Conflicts of Interest**

455 The authors have no conflicts of interest to declare.

456**Appendix A. Supplementary data**

457 Table s1-s2. Supplementary material related to this article can be found, in the
458 online version

459

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634

635**Table 1** Stand characteristics and soil properties of investigated forest in this study.

Stand characteristics	Dominant species	Species number	Density	Average DBH	Average tree height	Elevation	GPS location
	<i>Cyclobalanopsis glauca</i> ; <i>Lithocarpus glaber</i>	32	1696 plants hm ⁻²	8.8 cm	6.4 m	245-321 m	28°24'7.30" N, 113°18'46.11" E
Soil properties	Moisture content	Bulk density	Capillary porosity	Clay (<2 μm)	Sand (>50 μm)	pH	Field water-holding capacity
	29.2%	1.08 g cm ⁻³	14.0%	29.80%	21.97%	4.22	129.63 g kg ⁻¹

636DBH, diameter at breast height; N, north; E, east.

637**Table 2 Selected properties of the soil and the litters.**

Characteristics	Soil	Natural litter	Labeled litter
Annual litters amount (g m ⁻²)	-	98.42	-
MBC (mg kg ⁻¹)	686.03	-	-
MBN (mg kg ⁻¹)	30.42	-	-
MBC/MBN	27.8	-	-
TOC (g kg ⁻¹)	40.64	502.81	464.67
TN (g kg ⁻¹)	2.66	18.06	20.60
TOC/TN	15.0	28.7	22.7
¹⁵ N atom%	0.37	0.37	16.69

638 MBC, microbial biomass carbon; MBN, microbial biomass N; MBC/MBN, microbial biomass carbon to
639 nitrogen ratio; TOC, total organic carbon; TN, total nitrogen; TOC/TN, total organic carbon to nitrogen
640 ratio.

641 **Figure captions**

642 **Fig. 1** Study location

643 **Fig. 2** Concentrations of SON (a), $\text{NH}_4^+\text{-N}$ (b), $\text{NO}_3^-\text{-N}$ (c), and TN (d) in soil with litter
644 removal and litter retention. The shaded parts in the figure are the concentrations of the
645 following year. Bars represent the standard deviation ($n=3$) and are smaller than the symbol
646 when not visible. Asterisk designations indicate significant differences between different
647 treatments (*, $p < 0.05$; **, $p < 0.01$). P values of F test using one-way analysis of variance
648 (ANOVA) between sampling time in three treatments were also showed in figure (p_B , p
649 value in labeled litter addition; p_N , p value in natural litter addition; p_O , p value in litter
650 removal).

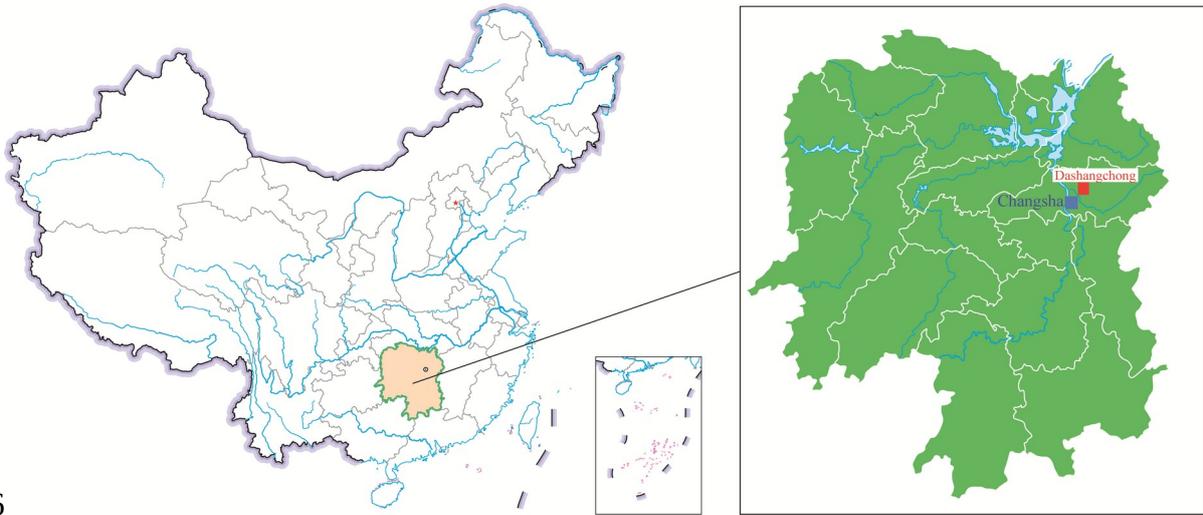
651 **Fig. 3** The ^{15}N abundances and ^{15}N mass of SON (a), $\text{NH}_4^+\text{-N}$ (b), $\text{NO}_3^-\text{-N}$ (c), and TN (d) in
652 soil with labeled litter addition. The shaded parts are the values of the following year. Bars
653 represent the standard deviation ($n=3$) and are smaller than the symbol when not visible. P
654 values of F test using one-way analysis of variance (ANOVA) between sampling time were
655 also showed in figure (p_a , p value in ^{15}N abundance; p_m , p value in ^{15}N mass).

656 **Fig. 4** ^{15}N recoveries of SON, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ (a), ^{15}N recoveries of TSN and TN (b) in
657 soils with labeled litter addition. Bars represent the standard deviation ($n=3$) and are smaller
658 than the symbol when not visible.

659 **Fig. 5** Relationships among the transformations of SON, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ in soil after
660 litter addition (a), and schematic diagram of more complex N-related processes (b). The
661 proportions of SON, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ and TN derived from litter N also shown in (a).
662 Bars represent the standard deviation ($n=3$) and are smaller than the symbol when not
663 visible.

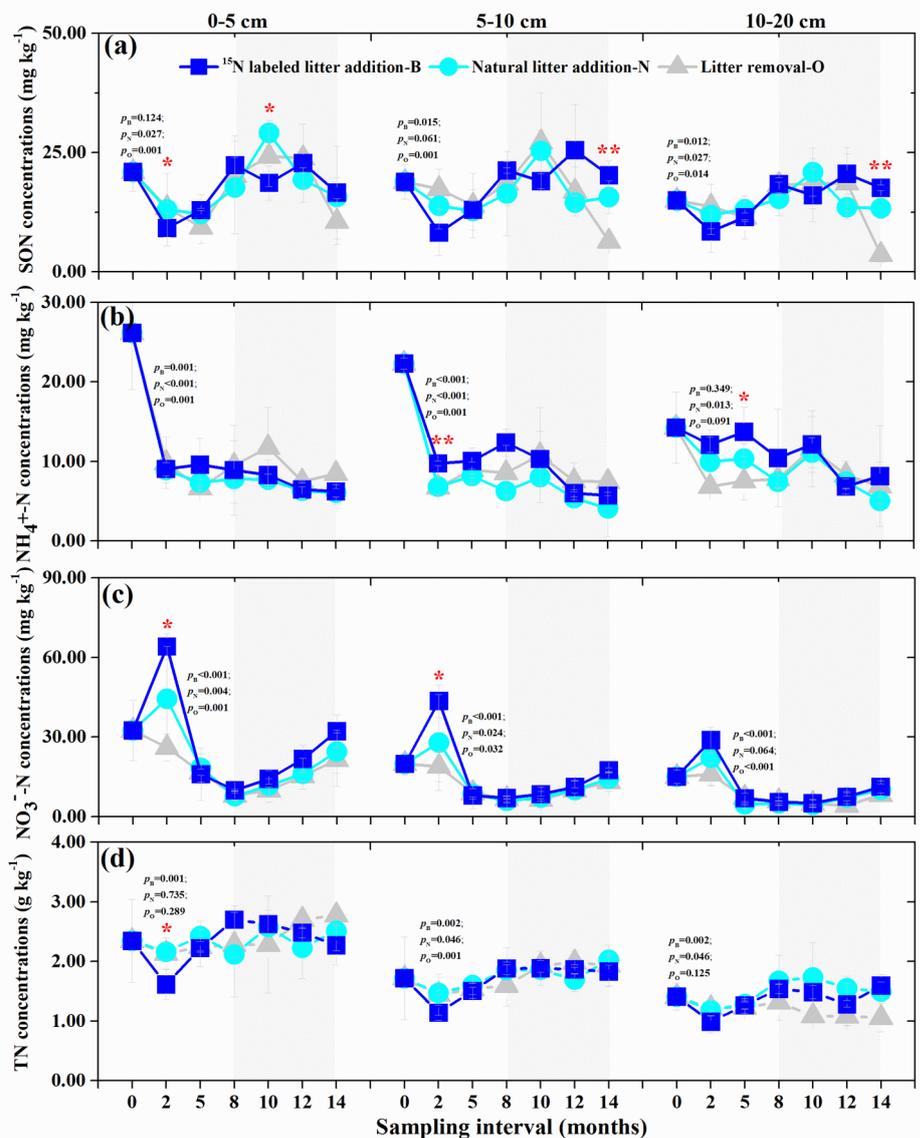
664

665 **Fig. 1** Study location



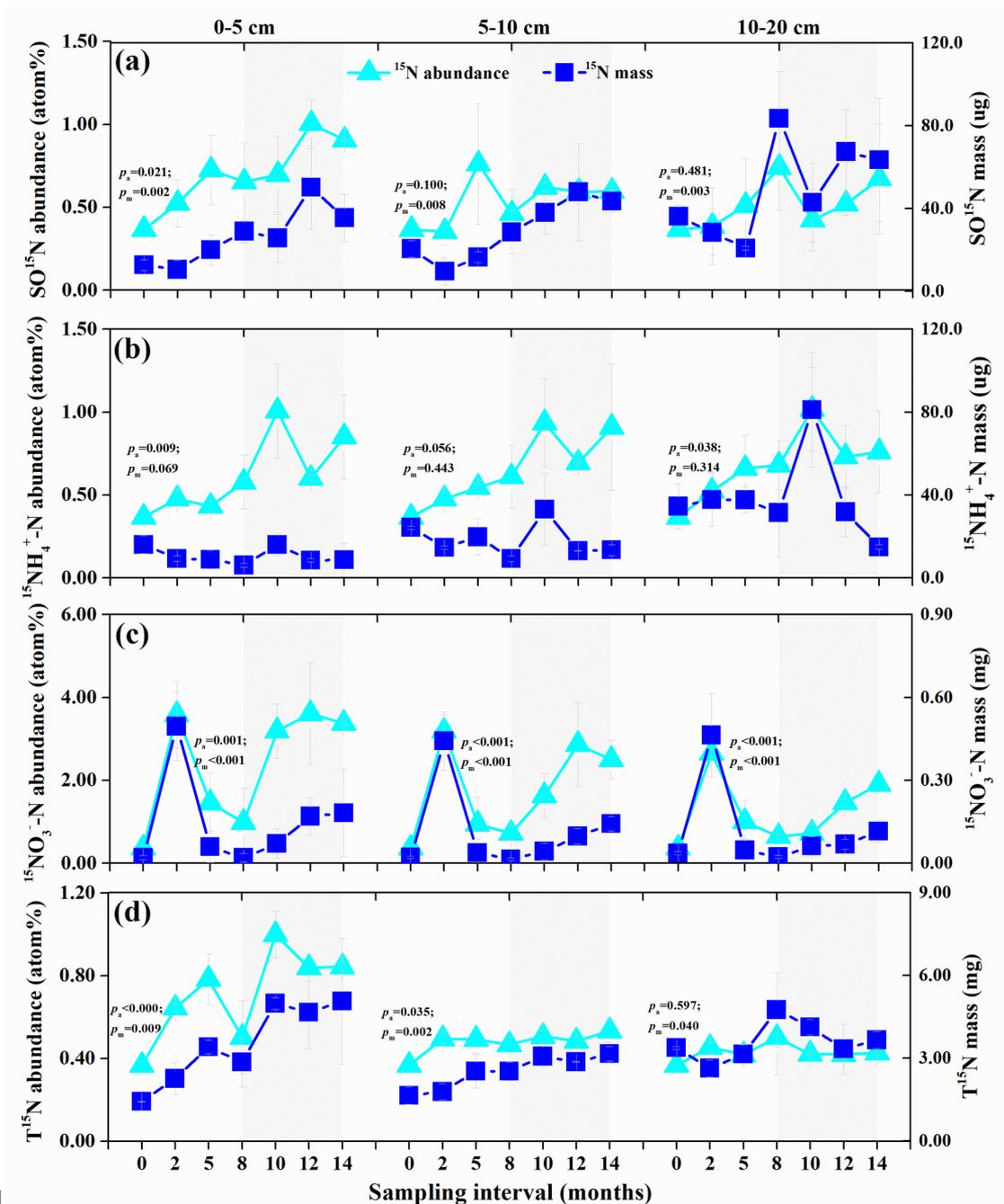
666

667**Fig. 2** Concentrations of SON (a), NH_4^+ -N (b), NO_3^- -N (c), and TN (d) in soil with litter
 668removal and litter retention. The shaded parts in the figure are the concentrations of the
 669following year. Bars represent the standard deviation ($n=3$) and are smaller than the symbol
 670when not visible. Asterisk designations indicate significant differences between different
 671treatments (*, $p < 0.05$; **, $p < 0.01$). P values of F test using one-way analysis of variance
 672(ANOVA) between sampling time in three treatments were also showed in figure (p_B , p
 673value in labeled litter addition; p_N , p value in natural litter addition; p_O , p value in litter
 674removal).



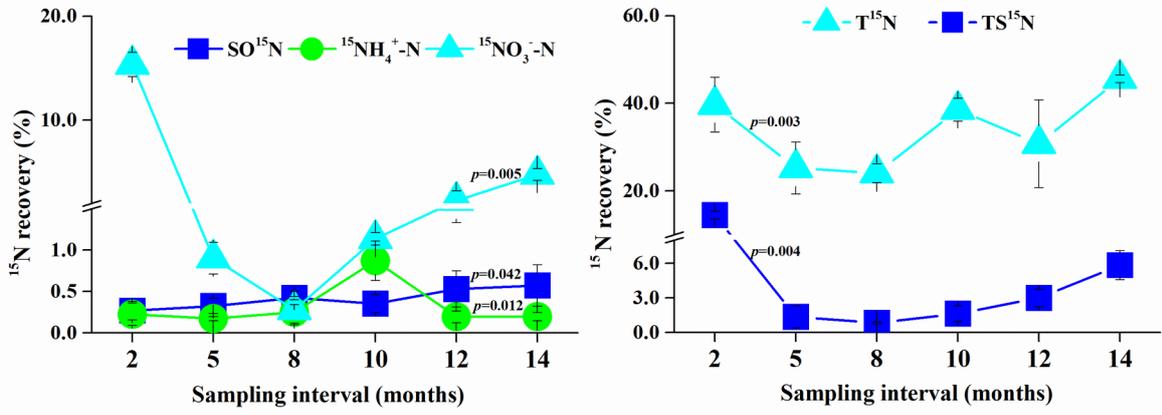
675

676 **Fig. 3** The ^{15}N abundances and ^{15}N mass of SON (a), $\text{NH}_4^+\text{-N}$ (b), $\text{NO}_3^-\text{-N}$ (c), and TN (d) in
 677 soil with labeled litter addition. The shaded parts are the values of the following year.
 678 Bars represent the standard deviation ($n=3$) and are smaller than the symbol when not visible. P
 679 values of F test using one-way analysis of variance (ANOVA) between sampling time were
 680 also showed in figure (p_a , p value in ^{15}N abundance; p_m , p value in ^{15}N mass).



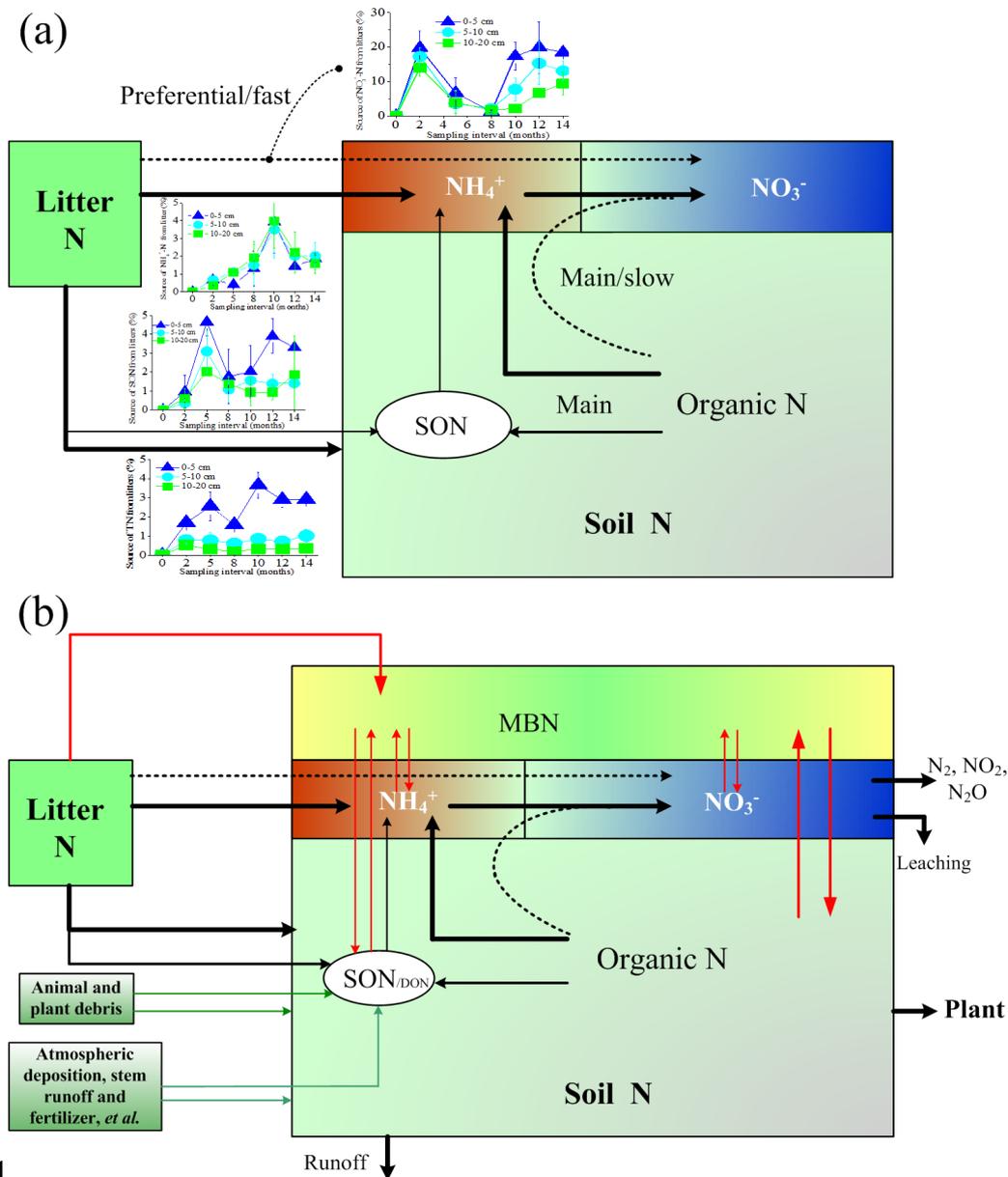
681

682 **Fig. 4** ^{15}N recoveries of SON , $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ (a), ^{15}N recoveries of TSN and TN (b) in
 683 soils with labeled litter addition. Bars represent the standard deviation ($n=3$) and are smaller
 684 than the symbol when not visible.



685

686 **Fig. 5** Relationships among the transformations of SON, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ in soil after
 687 litter addition (a), and schematic diagram of more complex N-related processes (b). The
 688 proportions of SON, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ and TN derived from litter N also shown in (a).
 689 Bars represent the standard deviation ($n=3$) and are smaller than the symbol when not
 690 visible.



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