



Title	The effect of snow reduction and <i>Eisenia japonica</i> earthworm traits on soil nitrogen dynamics in spring in a cool-temperate forest
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1 **The effect of snow reduction and *Eisenia japonica* earthworm traits on soil**  
2 **nitrogen dynamics in spring in a cool-temperate forest**

3

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17

18 **Abstract**

19 Due to spring climate warming, snowpack has been decreasing and coincidentally the  
20 snowmelt timing has been advancing in cool-temperate forests of northern Japan. To  
21 investigate the effect of advancing snowmelt in the end of snow season on soil nitrogen  
22 dynamics via overwintering earthworms, we conducted a snow removal field  
23 experiment at the end of snow season using mesocosms with and without earthworms.  
24 In this study, the difference in the effect of snow removal on soil nitrogen by the  
25 presence/absence and body size of *Eisenia japonica* was also tested in both early spring  
26 (April) and late spring (May). The snow removal did not influence the survival rate,  
27 body weight, or cast production of *E. japonica*. In early spring, while the snow removal  
28 increased nitrification significantly by 61%, the effect of the snow removal was similar  
29 irrespective of the earthworm treatments. This indicates that neither the presence of  
30 earthworms nor their body size matters for the effect of snow decrease in the end of  
31 snow season on soil nitrogen dynamics in early spring. In late spring, on the other hand,  
32 the effect of the snow removal was no longer significant on soil nitrogen dynamics,  
33 while ammonification and nitrification were significantly higher by 195% and by 50 %  
34 respectively in the mesocosms with adult earthworms compared to those without *E.*  
35 *japonica* earthworms. These findings indicate that the effect of snow decrease and

36 coincidental advancement of snowmelt timing in the end of snow season on soil

37 nitrogen appears only just after the snowmelt timing, alternatively, the effect of the

38 body size of earthworms on soil nitrogen dynamics is becoming predominant in late

39 spring in snowy cool-temperate forests.

40

41 Keywords: winter climate change, functional trait, off-season, ecosystem function,

42 carry-over effect

43

44

## 45 **1. Introduction**

46 At high latitudes, climate warming is pronounced (IPCC, 2013), especially during  
47 snow season compared to summer (Sturm et al., 2005). Due to climate warming, in  
48 snowy landscapes such as northern Japan, the maximum snowpack thickness and snow  
49 cover duration have decreased in the past 80 years largely due to the warm temperature  
50 in the end of snow season (Makoto et al., 2014). It has been suggested that the effect of  
51 climate change during winter is carried over to the following season (Xia et al., 2014)  
52 (especially spring; Blakinship et al., 2014; Brooks et al., 2003). Spring is important  
53 season for organism's growth and survival in temperate forests (e.g., Seiwa, 1999).  
54 Furthermore, nitrogen (N) is the determinant factor for many organisms and their  
55 ecosystem function in temperate forests (Fernández-Martínez et al., 2014). To achieve  
56 an accurate prediction of annual ecosystem functions in warming climates in snowy  
57 ecosystems, we need to understand how the decrease of snowpack in the end of snow  
58 season and coincidental decrease in snow cover duration influence soil N cycling in  
59 spring.

60 In cold biomes, soil organisms and soil N cycling are active under the snow cover  
61 (Christopher et al., 2008; Clein and Schimel, 1995; Isobe et al., 2018; Liao et al., 2015;

62 Saccone et al., 2013). This is largely because snowpack is highly porous and acts as an  
63 insulator for the soil against cold air temperatures; therefore, under the snowpack, the  
64 temperature is stable, just above 0°C (Shibata et al., 2013). As a result of active soil  
65 organisms, N dynamics in winter significantly contribute to the annual N budget. For  
66 instance, Kielland et al. (2006) reports 40% of the annual N mineralization occurs  
67 during the non-growing season in the boreal forests of US. In previous studies, winter  
68 climate change was shown to change the activity of soil microbes and, consequently,  
69 soil N dynamics in spring (Tan et al., 2014). However, some studies have shown that  
70 soil fauna act as an important factor in soil N processes in northern Japan (Hishi et al.,  
71 2014; Makoto et al., 2016). In particular, earthworms are one of the most dominant soil  
72 fauna in cool-temperate forests (Nakamura, 1972; Uchida et al., 2004) and have a  
73 significant influence on soil N dynamics in summer (Kawakami and Makoto, 2017;  
74 Makoto et al., 2016). Among the processes by which earthworm influence soil (e.g.,  
75 external mucus and urine released from the body and soil burrowing), feeding on litter  
76 and/or soil and subsequent cast production is one of the important pathways for  
77 influencing soil N dynamics (e.g., Bityutskii et al., 2002; Blouin et al., 2003; Scheu,  
78 1987). The presence of earthworms facilitates N cycling via soil and/or litter  
79 comminution and their digestive activity, thereby promoting N mineralization and the

80 subsequent nitrification by soil microbes through casts (Scheu, 1987). It is also known  
81 that climate warming enhances soil N dynamics by earthworms through the increase of  
82 cast production in northern Japanese forests (Makoto et al., 2016). On the other hand,  
83 this study focused on the effect of climate change on earthworm contributions to N  
84 cycling only in summer. Snowpack reduction results in more fluctuations of soil  
85 temperature in the beginning of snow season (Shibata et al., 2013). Previous studies  
86 revealed that the reduction of snowpack by warming climates could result in more  
87 frequent freeze-thaw cycles and have a negative influence on soil faunal abundance in  
88 the beginning and middle of snow season (Bokhorst et al., 2013, 2012; Huang, 2017;  
89 Templer et al., 2012). However, the relationship between the change in soil fauna by  
90 snow reduction and soil N dynamics has not been clarified, especially in the end of  
91 snow season. By considering the survival of earthworms in subzero temperatures in soil  
92 (Lumbricidae species: Caldelon et al., 2009; Holmstrup and Overgaard, 2007),  
93 earthworms could be active during winter, and therefore, snow reduction in the end of  
94 snow season would modify the soil N dynamics in spring via the change in earthworm  
95 activity.

96 The effect of climate change is known to differ depending on the functional traits of  
97 soil fauna (e.g., Dias et al., 2013). Bokhorst et al. (2012) reports that the body size of

98 soil fauna acts as an important response trait for winter climate change; smaller soil  
99 fauna is vulnerable to sudden snowmelt by extremely warm weather in the middle of  
100 winter. Furthermore, it is also known that the body size of soil fauna (e.g., Carrillo et  
101 al., 2011; Kaneda et al., 2017) can act as an effect trait that determines the magnitude of  
102 the effect of soil fauna on soil N cycling and aggregate formation. Such knowledge  
103 allows us to predict the body size of earthworms can act as both a response trait and an  
104 effect trait, which could determine the effect of snow reduction and coincidental  
105 advancing of snowmelt timing on earthworm survival and soil N via earthworms in  
106 spring.

107 In this study, by quantifying the impact of a shorter and shallower snow pack and  
108 presence of earthworms in spring on soil nitrogen dynamics, we aim to show the  
109 importance of winter climate change on the forestry via the change of soil N availability  
110 in snowy landscape. Based on the above-mentioned facts, we hypothesize that 1)  
111 snowpack reduction in the end of snow season negatively influences earthworm survival  
112 and their cast production and soil N dynamics in spring. On the other hand, we also  
113 hypothesize that 2) the effect of the change in snowpack on earthworms, their cast  
114 production and soil N dynamics might be larger when the large earthworm exist in soil.

115



116 **2. Materials and methods**

117 *2.1. Site description*

118 The field investigation was conducted in the nursery of Teshio Experimental Forest of  
119 Hokkaido University, located in northern Japan (44°57'N, 142°01'E). By conducting  
120 the experiment in the nursery within experimental forests, it is easier to link the  
121 outcome of the experiment to the consequence of snow reduction in the forests and  
122 forestry there. The mean annual temperature, mean minimum air temperature, and mean  
123 annual precipitation is approximately 6.0 °C, -25.0 °C, and 1100 mm, respectively  
124 (between 2010 to 2015). Thirty percent of the annual precipitation falls as snow from  
125 November to April. The soils of this region rarely freeze despite the cold air  
126 temperatures because the deep snow (the maximum snow depth is approximately 1.7 m)  
127 insulates the soil from the air at subzero temperatures throughout winter (Takagi et al.,  
128 2005). According to the Food and Agriculture Organization (FAO) classification, the  
129 soil type of the research site is Cambisol derived from sediment (see details of the soil  
130 properties in Makoto et al. (2016). In this area, in the past 80 years, the maximum snow  
131 depth has decreased approximately by 20cm due to climate warming and the snowmelt  
132 timing has been advancing approximately by two weeks in the end of snow season

133 (Makoto et al., 2014) and is predicted to decrease and advance in northern Japan

134 (Supplementary Fig. S1, Sapporo District Meteorological Observatory 2017).

135

## 136 2.2. Study design

137 We established 10 plots in Teshio Experimental Forest. All plots were apart from each

138 other by five meters. In each plot, three mesocosm were placed. Each mesocosm was 30

139 cm long, 30 cm wide and 20 cm deep. The mesocosm sidewalls were made of polyvinyl

140 chloride plates, and the upper and lower parts were made of nylon mesh (mesh opening:

141 2 mm). In the preliminary study, this mesh opening was sufficient to prevent the

142 juvenile and adult *E. japonica*, whose size was used in this study, to escape from

143 mesocosms. Each mesocosm contained 2.3 kg of wet soil (gravimetric moisture content

144 was about 31%) after sieving (with a 4.75 mm sieve) to homogenize and remove, fine

145 roots, gravel, and coarse woody debris were placed in each plot (a total of 30

146 mesocosms). In each plot, one of the three mesocosms contained 10 adult *E. japonica*,

147 one contained 10 juvenile *E. japonica*, and the other contained no *E. japonica*. *E.*

148 *japonica* is the most dominant geophagous earthworm species in northern Hokkaido

149 (Nakamura, 1972). The density of the earthworm in our study (111 individuals m<sup>-2</sup>) is

150 within the range of the field observation (0-216 individuals m<sup>-2</sup>) which we conducted in

151 our experimental forests. The average fresh body weight of the adult individuals was  
152  $0.80 \pm 0.18$  g, and that of the juvenile individuals was  $0.24 \pm 0.07$  g, which was the  
153 representative body weight respectively for adult and juvenile individuals in the  
154 experimental forest. The soil within the mesocosms was set to a depth of 15 cm so that  
155 the earthworms could live at a depth of approximately 10 cm, where most of the  
156 earthworms distributed in winter under snow in northern Hokkaido (Makoto and  
157 Kawakami, in press) and soil does not freeze throughout winter due to the heavy snow  
158 and its insulation (Shibata et al., 2016). The mesocosms was buried into the soil so that  
159 the bottom of the mesocosms reached to 15 cm deep in the site. The incubation was  
160 conducted from 27<sup>th</sup> November 2016, when the continuous snow cover starts in  
161 northern Hokkaido, to 23<sup>rd</sup> May 2017, when the forest plants start to develop leaves  
162 (Seiwa, 1999). At our site, snowmelt timing is generally from the middle of April to the  
163 beginning of May.

164

### 165 *2.3. Field manipulation*

166 To test the effect of snow reduction in the end of snow season, snow removal was  
167 conducted on 31<sup>st</sup> March, when the snowpack thickness was about 1.2 m. For half of the  
168 10 plots, we shoveled the snow about by 60 centimeters by caring not to disturb the

169 mesocosms by leaving approximately 30 centimeters of snow cover (Templer et al.,  
170 2012). A thirty centimeters of snow is sufficient to insulate soil against most of the  
171 freezing ambient temperatures in spring. As a result, the snowpack thickness was 1.2 m  
172 in the control plots and 0.3 m in the snow-removed plot on 31<sup>st</sup> March. In the snow-  
173 removed plots, the snow disappeared on 7<sup>th</sup> April, while the snow in the control plots  
174 disappeared on 21<sup>st</sup> April. In the two plots, the soil temperature was monitored at 5 cm  
175 depth from March to the end of May using temperature sensors (Ondotori Jr., T&D,  
176 Tokyo, Japan) (Fig. 1). We conducted the snow removal in this season to check the  
177 effect of snow reduction and coincidental advancing snowmelt season in the end of  
178 snow season on earthworm and soil N dynamics. The snow removal in the beginning of  
179 winter, which has been often conducted in previous studies (e.g. Groffman et al., 2001;  
180 Shibata et al., 2013), focuses on the effect of snow reduction and coincidental soil frost  
181 on soil microbiology especially in the beginning of winter. On the other hand, the  
182 reduction of snow and coincidental advancing of snowmelt timing in the end of snow  
183 season are more likely to occur in our region (Supplementary Fig.S1). Snow removal in  
184 the beginning of winter does not efficiently result in the reduction of snow in the end of  
185 winter because of the wind deposition of snow during winter. There was no difference  
186 of water contents between control plots (37.3%) and the plots with snow reduction

187 (38.4%) in the end of April and control plots (32.0%) and the plots with snow reduction  
188 (33.2%) in the end of May, respectively.

189

#### 190 *2.4. Earthworms*

191 At the beginning of the experiment, all Haplotaxida earthworms and their eggs were  
192 retrieved carefully by hand sorting method. The survival rate of *E. japonica* in each  
193 mesocosm was measured at the end of the incubation (23<sup>rd</sup> May 2017) by calculating  
194 the percentage of 10 surviving individuals which were added at the start of the  
195 experiment. Furthermore, the mean body weight (fresh weight) of *E. japonica* was also  
196 measured at the end of May after leaving the earthworms overnight in the laboratory  
197 without any feeding. The mean weight of each *E. japonica* was calculated by dividing  
198 the total weight of the earthworm population in each mesocosm by the number of  
199 individuals. Because the mesh of mesocosm was broken because of the heavy snow, the  
200 data of one mesocosm cannot be used for the analysis and consequently, the replication  
201 varied between 4 and 5 across the treatments. Because the sampling and measurement  
202 of earthworm requires severe disturbance on soil in mesocosms, we remained the  
203 earthworm untouched without any measurements of them until the end of experiment.

204

205 *2.5. Aggregate production*

206 We measured the cast produced by earthworm cast by conducting the dry sieving  
207 method (Blanchart, 1992). The soil was sieved to <4.75 mm before the incubation.  
208 Therefore, aggregates >4.75 mm were considered to be earthworm casts after the  
209 incubation. After the incubation, a portion of the soil (approximately 100 g) was  
210 obtained from the mesocosms, and the soil was separated into aggregates >4.75 mm  
211 (cast) using a 4.75 mm sieve. The casts were then weighed after they were dried in an  
212 oven at 75 °C for 1 week. There are factors other than earthworms that can make  
213 aggregates >4.75 mm, such as soil microorganisms. Thus, the earthworm-free  
214 mesocosms were used as control for these other sources of aggregates. The weight of  
215 aggregates was obtained by subtracting the earthworm-free mesocosms from those with  
216 worms. This gave the specific value associated with the worms. For the details of the  
217 sieving, please refer to the methods section in Kawakami and Makoto (2017).

218

219 *2.6. Soil nitrogen*

220 The net rates of  $\text{NH}_4^+$ -N production (ammonification) and  $\text{NO}_3$ -N production  
221 (nitrification) were calculated as the net changes in the soil pool for each ion during the  
222 incubation. Before and after the half-year incubation, eight grams of soil from each

223 mesocosm was shaken with 40 ml of 2 M KCl solution for one hour and filtered  
224 (Advantec 5B, Advantec Co Ltd., Tokyo, Japan). The concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$   
225 were determined using a continuous flow injection analyzer (AACS-4, BL-Tech Co.  
226 Ltd., Osaka, Japan). To check the effect of snow removal, we obtained the samples on  
227 25<sup>th</sup> April, soon after snow disappearance when the effect of snow manipulation on  
228 surface soil is known to be profound (Blakinship et al., 2014), and on 23<sup>rd</sup> May, when  
229 the plants in forest ecosystems starts to increase their activity after snow disappearance.  
230 We measured the ammonification and nitrification rates as the net change in  $\text{NH}_4^+$  and  
231  $\text{NO}_3^-$  concentration between 27<sup>th</sup> November and 25<sup>th</sup> April and between 25<sup>th</sup> April and  
232 23<sup>rd</sup> May 2017 as the value in early spring (soon after snow disappearance) and in late  
233 spring, one month after snow disappearance, respectively. Ideally, to check the  
234 influence of snow removal. we should have sampled the soil also just before the snow  
235 removal (end of March) to confirm the soil condition are similar across the mesocosms  
236 before the snow removal. However, not all snow can be removed to avoid the effect of  
237 direct disturbance of snow removal by shoveling on soil (Templer et al., 2012) and thus  
238 we assumed that there was no significant difference among the mesocosms before the  
239 snow removal, which we should be careful to interpret the data.  
240

241 *2.7. Statistical analysis*

242 After checking the normal distribution of the data with the Shapiro-Wilk test, we  
243 conducted a two-way analysis of variance (ANOVA). For the untransformed data that  
244 did not have a normal distribution, the data were log-transformed and analyzed with  
245 ANOVA. The explanatory variables were the snow removal treatment (control and  
246 snow removal condition) and earthworm condition (no earthworms, juvenile  
247 earthworms, and adult earthworms). Given the detection of a significant influence of the  
248 explanatory variables, Tukey's HSD multiple comparison tests were conducted to find  
249 the significant differences among the treatments. One mesocosm without earthworm  
250 under control snow condition was broken. Therefore, for statistical analysis, the data of  
251 earthworm and soil of this broken mesocosm were not included and thus replication of  
252 this treatment was 4, while that of other treatments was 5. All the statistical analyses  
253 were conducted using R software version 3.2.1. (R Developing Core Team, 2015).

254

255 **3. Results**

256 *3.1. Earthworm survival*

257 The snow removal did not influence the survival rate of *E. japonica* (Fig. 2a). The  
258 survival rate was significantly higher for adult *E. japonica* (between 10-100%) than for



259 juvenile *E. japonica* (between 10-60%) ( $p < 0.05$ ,  $F = 5.09$ , Fig. 2a). On the other hand,  
260 snow removal did not affect mean body weight of *E. japonica* (Fig. 2b).

261

### 262 3.2. Aggregates

263 The snow removal did not significantly influence the aggregate production by *E.*  
264 *japonica* in the end of the experiment (Fig. 3). The adult *E. japonica* produced 1.3 times  
265 more aggregates than the juvenile *E. japonica* on average ( $p < 0.001$ ,  $F = 21.51$ , Fig. 3).

266

### 267 3.3. Soil nitrogen dynamics

268 In early spring, the snow removal resulted in 61% increase of the nitrification rate  
269 ( $p < 0.05$ ,  $F = 4.85$ , Fig. 4b), while there was no influence on the ammonification rate (Fig.  
270 4a). On the other hand, there was no significant influence of the earthworm treatment on  
271 either the ammonification or the nitrification rates (Fig. 4a, b) in early spring.

272 Furthermore, no significant interactive effect of the earthworm and snow removal  
273 treatments on the N dynamics was detected in this period.

274 In late spring, the effect of the snowmelt treatment was no longer significant on the  
275 ammonification and nitrification rates (Fig. 5a, b). On the other hand, in the mesocosms  
276 with adult *E. japonica*, 195% higher rates of ammonification ( $p < 0.05$ ,  $F = 4.86$ , Fig. 5a)

277 and 50% higher rates of nitrification ( $p < 0.05$ ,  $F = 4.85$ , Fig. 5b) were observed as  
278 compared to the mesocosms without earthworm. No significant interactive effect of the  
279 earthworm and snow removal treatments on the N dynamics was detected in late spring  
280 (Fig. 5a, b).

281

#### 282 **4. Discussion**

283 Contrary to our expectations, our results indicate that the importance of the  
284 earthworm presence was small to understand the consequence of snow removal on soil  
285 N dynamics from winter to early spring (Fig. 4). On the other hand, in a previous study,  
286 the milder winter is implicated to activate the earthworm in the temperate forests of US  
287 (Eisenhauer et al., 2014). However, no influence of snow reduction on the survival rate,  
288 body weight and aggregate production demonstrate that reduction of snow and earlier  
289 snowmelt does not likely to activate the earthworm in Japanese temperate forests. For  
290 the microarthropods, snow reduction in the beginning of winter was found to decrease  
291 the abundance of soil arthropods due to the increase in soil frost in cool-temperate  
292 forests (Templer et al., 2012). In our study, however, the snow reduction resulted in the  
293 early increase of the soil temperature and did not cause any soil frost (Fig. 1). In the  
294 Hokkaido region, the deep winter snowpack (average 1.5 m deep; Makoto et al., 2014)

295 takes many days to disappear (until the end of April at low altitudes), and there is  
296 usually sufficient snow to insulate the soils and earthworms from air temperature  
297 below-freezing throughout the period, making the average soil temperatures during the  
298 snowmelt period above zero (Japan Meteorological Agency, 2017). Therefore, in our  
299 study, the two-week advancement of snow disappearance by snow reduction in the end  
300 of snow season did not result in the exposure of the soil and earthworms to below-  
301 freezing temperatures. Furthermore, it is also possible that even the high rate of  
302 earthworm survival and their overwintering are not influential on the soil N dynamics in  
303 northern Japan. To our knowledge, the influence of overwintering earthworms on soil N  
304 in the early stages has not been clarified, and our study indicates that the effect is  
305 negligible. In a previous study, the effect of soil warming was significantly positive on  
306 the soil N dynamics due to the increase of aggregate production by earthworm in  
307 summer (Makoto et al., 2016). However, our study suggested that the enhancement of  
308 soil N dynamics by earthworms under warming might occur only during the growing  
309 season. In early spring, the enhanced nitrification by snow reduction could be due to the  
310 earlier exposure of soil to warmer air temperatures (Fig. 1) and the coincidental increase  
311 of soil microorganisms, which was reported in a previous study (Melillo et al., 2011).

312 Earthworm body size was expected to act as both a response trait to snow decrease  
313 (as observed in meso-fauna, Bokhorst et al., 2012) and an effect trait on soil N  
314 dynamics. However, our study found that there was no effect of the snow removal on  
315 the survival rate of the earthworms (Fig. 2), implying that the power of body weight as a  
316 response trait to snow reduction is, to a certain extent, small for predicting the effect of  
317 snow removal in the end of snow season. Body size acted as an important predictor of  
318 soil faunal abundance after snow reduction by an extreme warming pulse in the arctic  
319 tundra in the middle of winter (Bokhorst et al., 2012). In that case, the frost event  
320 occurred due to snowmelt after an extreme warming pulse, and there was no frost event  
321 after snowmelt in our study. This implies that the body size of soil fauna could be used  
322 as an important response trait, not for the change in snow amount, but for the  
323 coincidental changes in the soil freeze-thaw cycles. The knowledge about the  
324 relationship between the functional traits of overwintering organisms and winter climate  
325 change is still limited (Cornelissen and Makoto, 2014), which should be tested in future  
326 studies. As described in Materials and Methods section, there was no difference of  
327 water contents between control pots and the plots with snow removal both in the end of  
328 April and in the end of May. We are not sure why there was no difference in soil water  
329 contents between control plots and the plots with snow reduction. Based on these facts,

330 we assume that the obtained results are not due to the difference of soil water regime.  
331 Further manipulation studies or analysis such as structural equation modeling are  
332 necessary to clarify the underlying mechanisms of similar soil water regime between the  
333 two conditions.

334 The survival rate of the adult *E. japonica* was generally higher than that of the  
335 juvenile *E. japonica* (Fig. 2), which suggests that body size acts as an important  
336 determinant for overwinter survival in ambient conditions. Chemical traits, such as  
337 glycogen reserves and glucose mobilization (as a cryoprotectant), are known to enhance  
338 the survival rate of earthworms (Caldelon et al., 2009; Holmstrup and Overgaard, 2007).  
339 In addition to the chemical properties, our study suggests that morphological traits are  
340 also important for determining the survival of earthworms during the snow season. On  
341 the other hand, in our study, the difference of the body size is obtained by the difference  
342 of growth stage of earthworm, because both juvenile and adult earthworm existed in the  
343 soil abundantly in the end of fall (beginning of winter) and the distinct difference of  
344 body size existed between the two groups. Previous studies show that adult earthworm  
345 is more resistance to the environmental stress than juvenile worms (e.g., Spurgeon and  
346 Hopkin 1996). We should be careful to identify whether the difference survival rate is  
347 due to the difference of body size or growth stage. In the future study, we should

348 conduct an experiment to understand the difference of survival rate is due either to the  
349 size or growth stage of the earthworm, which is little understood so far.

350 Although body size is limited as a response trait, its usefulness as an effect trait on  
351 soil N dynamics was validated in late spring. The adult earthworms enhanced both the  
352 ammonification and nitrification in late spring as compared to the condition without  
353 earthworm (Fig. 5), while juveniles did not make any significant change. Earthworms  
354 are known to accelerate soil N mineralization through the comminution of organic  
355 matter and the production of soil aggregates with the aid of their gut bacteria (Makoto et  
356 al., 2016). In fact, the adult *E. japonica* produced more aggregates than the juvenile *E.*  
357 *japonica* in spring (Fig. 3). Adult *E. japonica* can eat more soil, which could have  
358 resulted in the increased production of soil aggregates in spring (Fig. 3). However, the  
359 influence of soil aggregates is small in early spring considering the absence of a  
360 significant effect on the N dynamics in that season (Fig. 3). Again, this could be  
361 because the earthworms are alive under the snow, and their activity is not sufficiently  
362 high to modify the soil condition in early spring. As a result, the effect of the earthworm  
363 trait was not significant (although we did not check the aggregates and survival of the  
364 earthworms immediately following snow disappearance to prevent the disturbance of  
365 the mesocosms). In the previous study, Isobe et al. (2018) indicated the activity of soil

366 microbe as N sink during dormant season is important for the annual N cycling in  
367 temperate forests of northern Japan. Our study indicate earthworm, the most dominant  
368 soil fauna, do not have such function during the snowy season in temperate forests.

369

370

## 371 **5. Conclusion**

372 Our results indicate that, under the warming climate, the effect of snow decrease in  
373 the end of snow season does not last long in spring, and alternatively, the effect of  
374 earthworm body size on soil N dynamics is becoming predominant in the spring of  
375 snowy cool-temperate forests. In temperate forests, soil N availability is important for  
376 tree seedlings especially in spring due to the high demand of N for the development of  
377 new growth and leaf flush (Fotelli et al., 2004). Our study revealed that both snow  
378 reduction and earthworm presence in spring could have substantial influence on planted  
379 seedlings in temperate region. Future studies of the season-dependent importance of  
380 functional traits and their relationship with climate change are needed to predict the  
381 dynamics of soil fauna and their relationship with soil N dynamics in forest soils across  
382 the seasons.

383

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389



390 **References**

- 391 Bityutskii, N., Kaidun, P., Yakkonen, K., 2016. Can earthworms alleviate nutrient  
392 disorders of plants subjected to calcium carbonate excess? *Appl. Soil Ecol.* 98, 20-  
393 29.
- 394 Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussard, L., Butt, K.R., Dai, J.,  
395 Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.-J., 2013. A review of  
396 earthworm impact on soil function and ecosystem services. *Eur. J. Soil Sci.* 64,  
397 161–182.
- 398 Blanchart, E., 1992. Restoration by earthworms (Megascolecidae) of the  
399 macroaggregate structure of a destructured savanna soil under field conditions.  
400 *Soil Biol. Biochem.* 24, 1587–1594.
- 401 Blakinsip, J.C., Meadows, M.W., Lucas, R.G., Hart, S.C., 2014. Snowmelt timing  
402 alters shallow but not deep soil moisture in the Sierra Nevada. *Water Resour. Res.*  
403 50, 1448–1456.
- 404 Bokhorst, S., Phoenix, G.K., Bjerke, J.W., Callaghan, T.V., Huyer-Brugman, F., Berg,  
405 M.P., 2012. Extreme winter warming events more negatively impact small rather  
406 than large soil fauna: Shift in community composition explained by traits not  
407 taxa. *Glob. Change Biol.* 18, 1152–1162.

408 Bokhorst, S., Metcalfe, D.B., Wardle, D.A., 2013. Reduction in snow depth negatively  
409 affects decomposers but impact on decomposition rates is substrate  
410 dependent. *Soil Biol. Biochem.* 62, 157–164.

411 Brooks, P.D., Williams, M.W., Schmidt, S.K., 1998. Inorganic nitrogen and microbial  
412 biomass dynamics before and during spring snowmelt. *Biogeochemistry* 43, 1–15.

413 Calderon, S., Holmstrup, M., Westh, P., Overgaard, J., 2009. Dual roles of glucose in  
414 the freeze-tolerant earthworm *Dendrobaena octaedra*: cryoprotection and fuel for  
415 metabolism. *J. Exp. Biol.* 212, 859-866.

416 Carrillo, T., Ball, B.A., Bradford, M.A., Jordan, C.F., Molina, M., 2011. Soil fauna alter  
417 the effects of litter composition on nitrogen cycling in a mineral soil. *Soil Biol.*  
418 *Biochem.* 43, 1440-1449.

419 Christopher, S.F., Shibata, H., Ozawa, M., Nakagawa, Y., Mitchell, M.J., 2008. The  
420 effect of soil freezing on N cycling: comparison of two headwater subcatchments  
421 with different vegetation and snowpack conditions in the northern Hokkaido  
422 Island of Japan. *Biogeochemistry* 88, 15–30.

423 Clein, J.S., Schimel, J.P., 1995. Microbial activity of tundra and taiga soils at sub-zero  
424 temperatures. *Soil Biol. Biochem.* 27, 1231–1234.

425 Cornelissen, J.H.C., Makoto, K., 2014. Winter climate change, plant traits and nutrient  
426 and carbon cycling in cold biomes. *Ecol. Res.* 29, 517-527.

427 Dias, A.T., Krab, E.J., Marien, J., Zimmer, M., Cornelissen, J.H., Ellers, J., Wardle,  
428 D.A., Berg, M.P., 2013. Traits underpinning desiccation resistance explain  
429 distribution patterns of terrestrial isopods. *Oecologia* 172, 667–677.

430 Eisenhauer, N., Stefanski, A., Fisichelli, N.A., Rice, K., Rich, R., Reich, P.B., 2014.  
431 Warming shifts ‘worming’: effects of experimental warming on invasive  
432 earthworms in northern North America. *Sci. Rep.* 4, 6890.

433 Fernández-Martínez, M., Vicca, S., Janssens, I.A., Sardans, J., Luysaert, S., Campioli,  
434 M., Chapin, F.S. III, Ciais, P., Malhi, Y., Obersteiner, M., Papale, D., Piao,  
435 S.L., Reichstein, M., Rodà, F., Peñuelas, J., 2014. Nutrient availability as the key  
436 regulator of global forest carbon balance. *Nat. Clim. Chang.* 4, 471–476.

437 Fotelli, M.N., Rienks, M., Rennenberg, H., Gessler, A., 2004. Climate and forest  
438 management affect <sup>15</sup>N-uptake, N balance and biomass of European beech  
439 seedlings. *Trees* 18, 157–166.

440 Hishi, T., Urakawa, R., Tashiro, N., Maeda, Y., Shibata, H., 2014. Seasonality of  
441 factors controlling N mineralization rates among slope positions and aspects in

442 cool-temperate deciduous natural forests and larch plantations. *Biol. Fert. Soils* 50,  
443 343-356.

444 Holmstrup, M., Overgaard, J., 2007, Freeze tolerance in *Aporrectodea caliginosa* and  
445 other earthworms from Finland. *Cryobiology* 55, 80-86.

446 Huang, J., 2017. Presence of snow coverage and its thickness affected the mortality of  
447 overwintering pupae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae).  
448 *Int. J. Biometeorol.* 61, 709–718.

449 IPCC, 2013. *Climate Change 2013: The Physical Science Basis. Contribution of*  
450 *Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on*  
451 *Climate Change.* Cambridge University Press, Cambridge, UK and New York, NY,  
452 USA.

453 Isobe, K., Oka, H., Watanabe, T., Tateno, R., Urakawa, R., Liang, C., Senoo, K.,  
454 Shibata, H., 2018. High soil microbial activity in the winter season enhances  
455 nitrogen cycling in a cool-temperate deciduous forest. *Soil Biol. Biochem.* 124, 90-  
456 100.

457 Japan Meteorological Agency, 2017. <http://www.jma.go.jp/jma/indexe.html>. Data  
458 accessed on 1st April 2017.

459 Kaneda, S., Yonemura, S., Sakurai, G., 2017. Earthworm population is a significant  
460 regulator of rate of formation of soil aggregates: a case study in *Eisenia japonica*  
461 (Michaelsen, 1892). *Edaphologia* 100, 31-36.

462 Kawakami, T., Makoto, K., 2017. Does an earthworm species acclimatize and/or adapt  
463 to soil calcium conditions? The consequence of soil nitrogen mineralization in  
464 forest soil. *Ecol. Res.* 32, 603-610.

465 Kielland, K., Olson, K., Ruess, R.W., Boone, R.D., 2006. Contribution of winter  
466 processes to soil nitrogen flux in taiga forest ecosystems. *Biogeochemistry* 81,  
467 349–360.

468 Liao, S., Yang, W., Tan, Y., Peng, Y., Li, J., Tan, B., Wu, F., 2015. Soil fauna affects  
469 dissolved carbon and nitrogen in foliar litter in alpine forest and alpine  
470 meadow. *PLoS One* 10, e0139099.

471 Makoto, K., Arai, M., Kaneko, N., 2014. Change the menu? Species-dependent feeding  
472 responses of millipedes to climate warming and the consequences for plant-soil  
473 nitrogen dynamics. *Soil Biol. Biochem.* 72, 19-25.

474 Makoto, K., Kajimoto, T., Koyama, L., Kudo, G., Shibata, H., Yanai, Y., Cornelissen,  
475 J.H.C., 2014. Winter climate change in plant-soil system: summary of recent  
476 findings and future perspective. *Ecol. Res.* 29, 593-606.

477 Makoto, K., Minamiya, Y., Kaneko, N., 2016. Differences in soil type drive the  
478 intraspecific variation in the responses of an earthworm species and, consequently,  
479 tree growth to warming. *Plant. Soil* 404, 209-218.

480 Makoto, K., Kawakami, T. (in press) Earthworms under 1 m of snow: the seasonal  
481 dynamics of earthworm abundance in cool-temperate forests with heavy  
482 snowfall. *Edaphologia*.

483 Melillo, J.M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E.,  
484 Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhou, Y.M., Tang,  
485 J., 2011. Soil warming, carbon–nitrogen interactions, and forest carbon  
486 budgets. *Proc. Natl. Acad. Sci. USA* 108, 9508–9512.

487 Nakamura, Y., 1972. Ecological studies on the family Lumbricidae from Hokkaido I.  
488 Ecological distribution. *Jpn. Soc. Appl. Entomol. Zool.* 16, 18–23. (in Japanese  
489 with English summary)

490 R Developing Core Team, 2015. R: A language and environment for statistical  
491 computing. R foundation for Statistical Computing, Vienna, Austria. ISBN3-  
492 900051-07-0, URL <http://www.R-project.org>.

493 Saccone, P., Morin, S., Baptist, F., Bonneville, J.M., Colace, M.P., Domine, F.,  
494 Faure, M., Geremia, R., Lochet, J., Poly, F., Lavorel, S., Clément, J.C., 2013. The

495 effects of snowpack properties and plant strategies on litter decomposition during  
496 winter in subalpine meadows. *Plant. Soil* 363, 215-229.

497 1. Sapporo District Meteorological Observatory, 2017. Climate change in Hokkaido-  
498 past 120 years and future prediction 2<sup>nd</sup> edition (in Japanese). URL:  
499 <http://www.jma-net.go.jp/sapporo/tenki/kikou/kikohenka/ver2/report.pdf>. Data  
500 accessed on 1st April 2017.

501 Seiwa, K., 1999. Changes in leaf phenology are dependent on tree height in *Acer mono*,  
502 a deciduous broad-leafed tree. *Ann. Bot.* 83, 355–361.

503 Scheu, S., 1987. The influence of earthworm (Lumbricidae) on the nitrogen dynamics in  
504 the soil litter system of a deciduous forest. *Oecologia* 72, 197–201.

505 Shibata, H., Hasegawa, Y., Watanabe, T., Fukuzawa, K., 2013. Impact of snowpack  
506 decrease on net nitrogen mineralization and nitrification in forest soil of northern  
507 Japan. *Biogeochemistry* 116, 69-82.

508 Spurgeon, D.J., Hopkin, S.P., 1996. Effects of metal-contaminated soils on the growth,  
509 sexual development, and early cocoon production of the earthworm *Eisenia fetida*,  
510 with particular reference to Zinc. *Ecotox. Environ. Safe.* 35, 86-95.

511 Sturm, M., Schimel, J., Michaelson, G., Welker, J.M., Oberbauer, S.F., Liston, G.E.,  
512 Fahnestock, J., Romanovsky, V., 2005. Winter biological processes could help  
513 convert arctic tundra to shrubland. *Bioscience* 55, 17–26.

514 Takagi, K., Nomura, M., Ashiya, D., Takahashi, H., Sasa, K., Fujinuma, Y., Shibata, H.,  
515 Akibayashi, Y., Koike, T., 2005. Dynamic carbon dioxide exchange through  
516 snowpack by wind-driven mass transfer in a conifer-broadleaf mixed forest in  
517 northernmost Japan. *Global Biogeochem. Cy.* 19, GB2012.

518 Tan, B., Wu, F., Yang, W., He, X., 2014. Snow removal alters soil microbial biomass  
519 and enzyme activity in a Tibetan alpine forest. *Appl. Soil Ecol.* 76, 34-41.

520 Templer, P.H., Schiller, A.F., Fuller, N.W., Soggi, A.M., Campbell, J.L., Drake, J.E.,  
521 Kunz, T.H., 2012. Impact of a reduced winter snowpack on litter arthropod  
522 abundance and diversity in a northern hardwood forest ecosystem. *Biol. Fert. Soils*  
523 48, 413–424.

524 Uchida, T., Kaneko, N., Ito, M., Futagami, K., Sasaki, K., Sugimoto, A., 2004. Analysis  
525 of the feeding ecology of earthworms (Megascolecidae) in Japanese forests using  
526 gut content fractionation and d15N and d13C stable isotope natural abundances.  
527 *Appl. Soil Ecol.* 27, 153–163.



528 Xia, J., Chen, J., Piao, S., Ciais, P., Luo, Y., Wan, S., 2014. Terrestrial carbon cycle

529 affected by non-uniform climate warming, *Nat. Geosci.* 7, 173–180.

530

531 **Legends to figures**

532 **Fig. 1.** Air temperature (a) and soil temperature dynamics at 5cm depth (b) from 1<sup>st</sup>  
533 March to 31<sup>st</sup> May 2017 monitored in a site of Teshio Experimental Forest where we  
534 conducted a snow removal treatment. CON = without snow manipulation (control), SM  
535 = with snow manipulation.

536

537 **Fig. 2.** The effect of snow reduction and earthworm treatment on the survival rate of  
538 earthworm (a) and the individual body weight of earthworm (b) at the end of the  
539 experiment (late spring). Juvenile = with juvenile earthworm, Adult = with adult  
540 earthworm, CON = without snow manipulation (control), SM = with snow  
541 manipulation. Different letters mean the presence of statistically significant difference  
542 analyzed with ANOVA with Tukey HSD test ( $p < 0.05$ ). The asterisks show the level of  
543 significance by two-way ANOVA: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . n.s. indicates the  
544 lack of significant difference among the treatments.

545

546 **Fig. 3.** The effect of snow reduction and earthworm treatment on the amount of soil  
547 aggregate produced by earthworm at the end of the experiment (late spring). Juvenile =  
548 with juvenile earthworm, Adult = with adult earthworm, CON = without snow

549 manipulation (control), SM = with snow manipulation. Different letters mean the  
550 presence of statistically significant difference analyzed with ANOVA with Tukey HSD  
551 test ( $p < 0.05$ ). The asterisks show the level of significance by two-way ANOVA: \* $p <$   
552  $0.05$ , \*\* $p < 0.01$ , \* $p < 0.001$ . n.s. indicates the lack of significant difference among the  
553 treatments.

554

555 **Fig. 4.** The effect of snow reduction and earthworm treatment on ammonification rate  
556 (a) and nitrification rate (b) in early spring. No = without earthworm, Juvenile = with  
557 juvenile earthworm, Adult = with adult earthworm, CON = without snow manipulation  
558 (control), SM = with snow manipulation. Different letters mean the presence of  
559 statistically significant difference analyzed with ANOVA with Tukey HSD test  
560 ( $p < 0.05$ ). n.s. means the absence of statistically significant influence of the treatment.

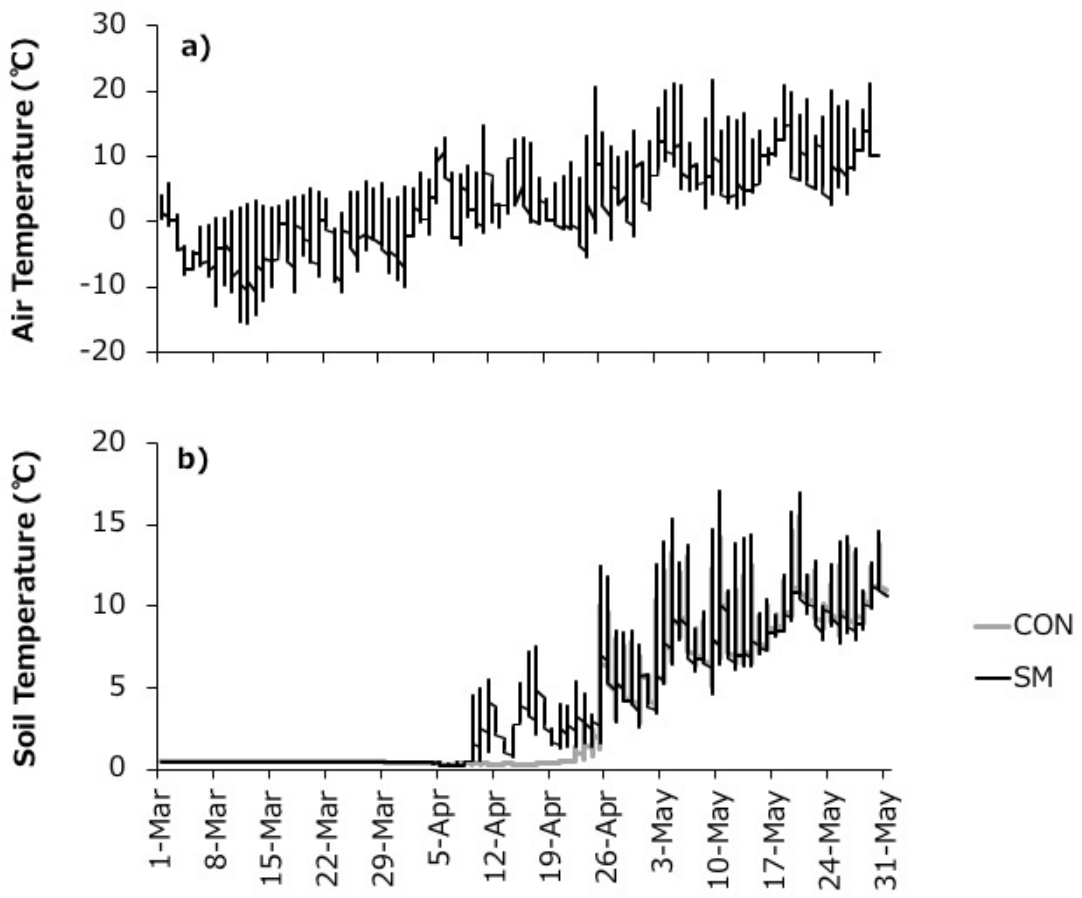
561 The asterisks show the level of significance by two-way ANOVA: \* $p < 0.05$ , \*\* $p <$   
562  $0.01$ , \* $p < 0.001$ . n.s. indicates the lack of significant difference among the treatments.

563

564 **Fig. 5.** The effect of snow reduction and earthworm treatment on ammonification rate  
565 (a) and nitrification rate (b) in late spring. No = without earthworm, Juvenile = with  
566 juvenile earthworm, Adult = with adult earthworm, CON = without snow manipulation

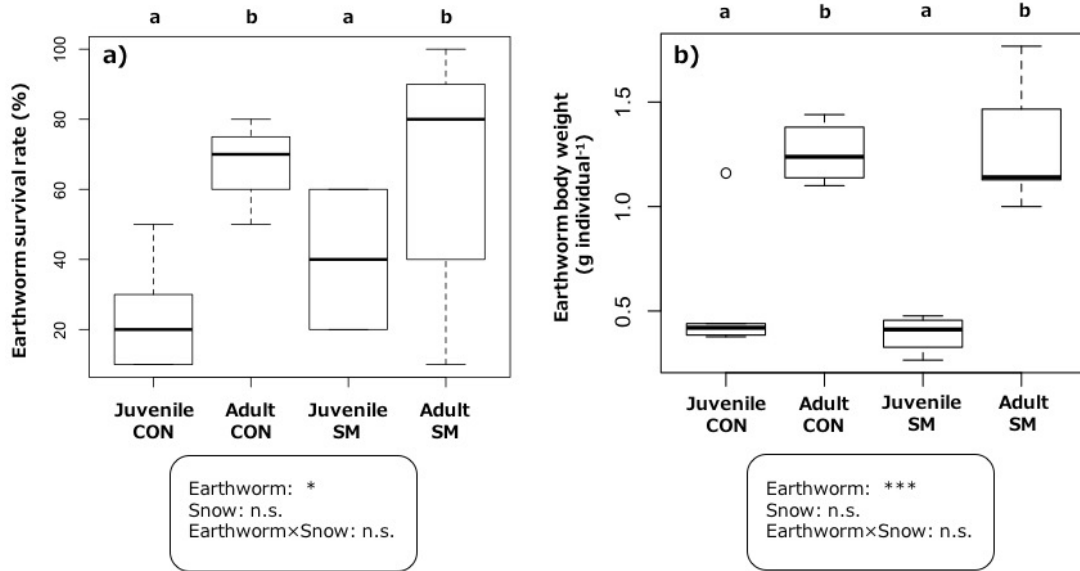
567 (control), SM = with snow manipulation. Different letters mean the presence of  
568 statistically significant difference analyzed with ANOVA with Tukey HSD test  
569 ( $p < 0.05$ ). The asterisks show the level of significance by two-way ANOVA: \* $p < 0.05$ ,  
570 \*\* $p < 0.01$ , \* $p < 0.001$ . n.s. indicates the lack of significant difference among the  
571 treatments.  
572

573 Fig.1

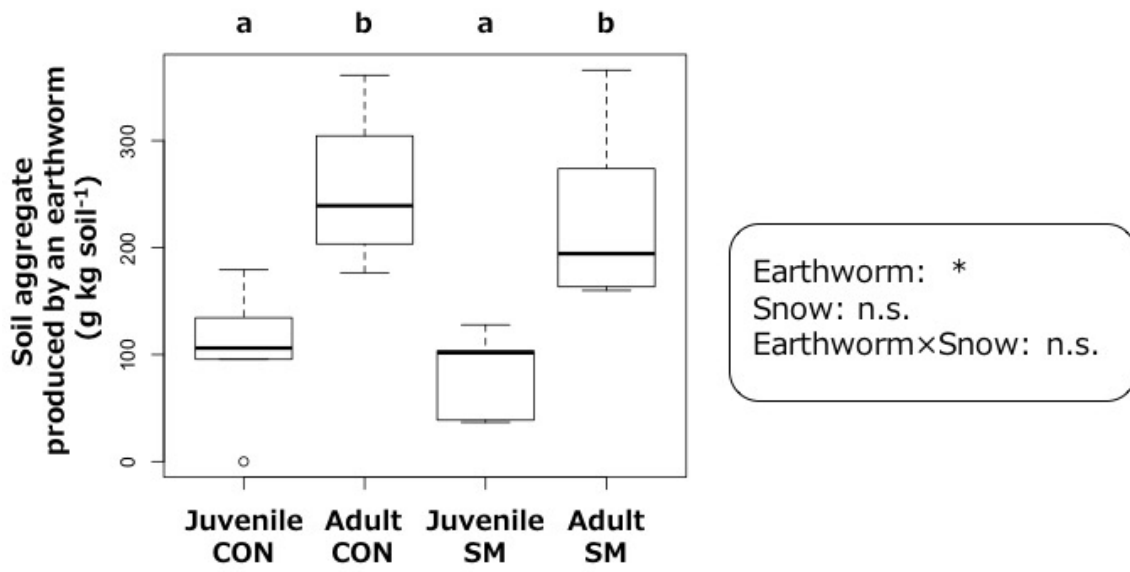


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578 Fig. 3

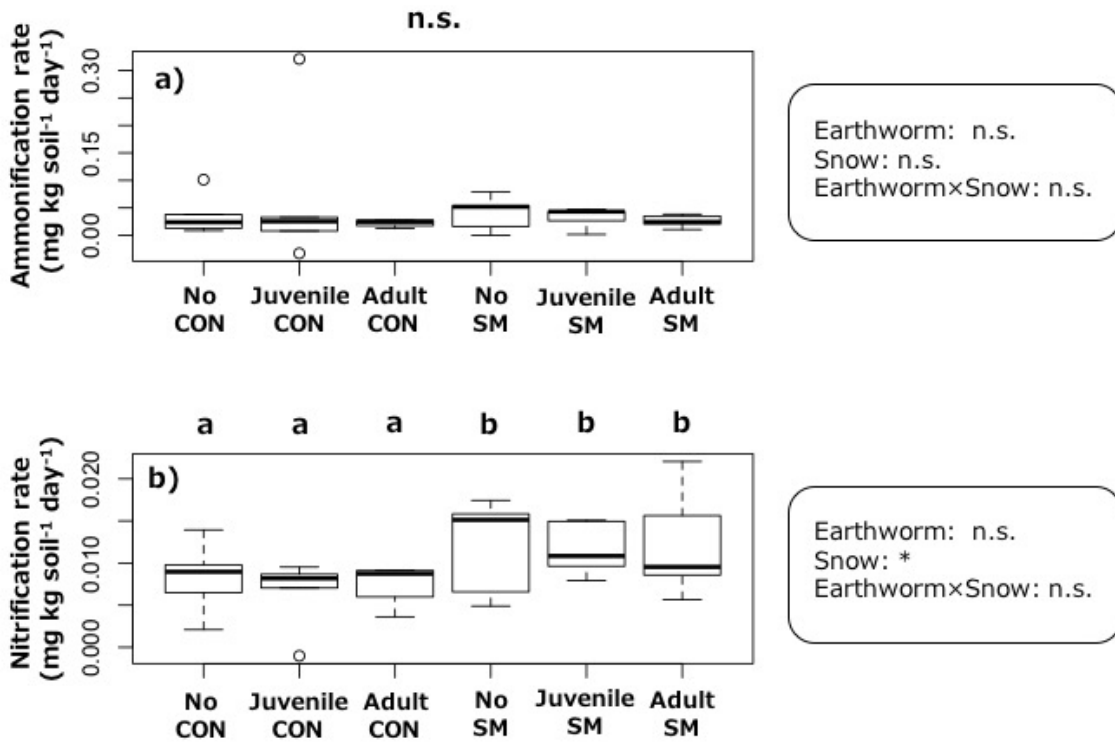


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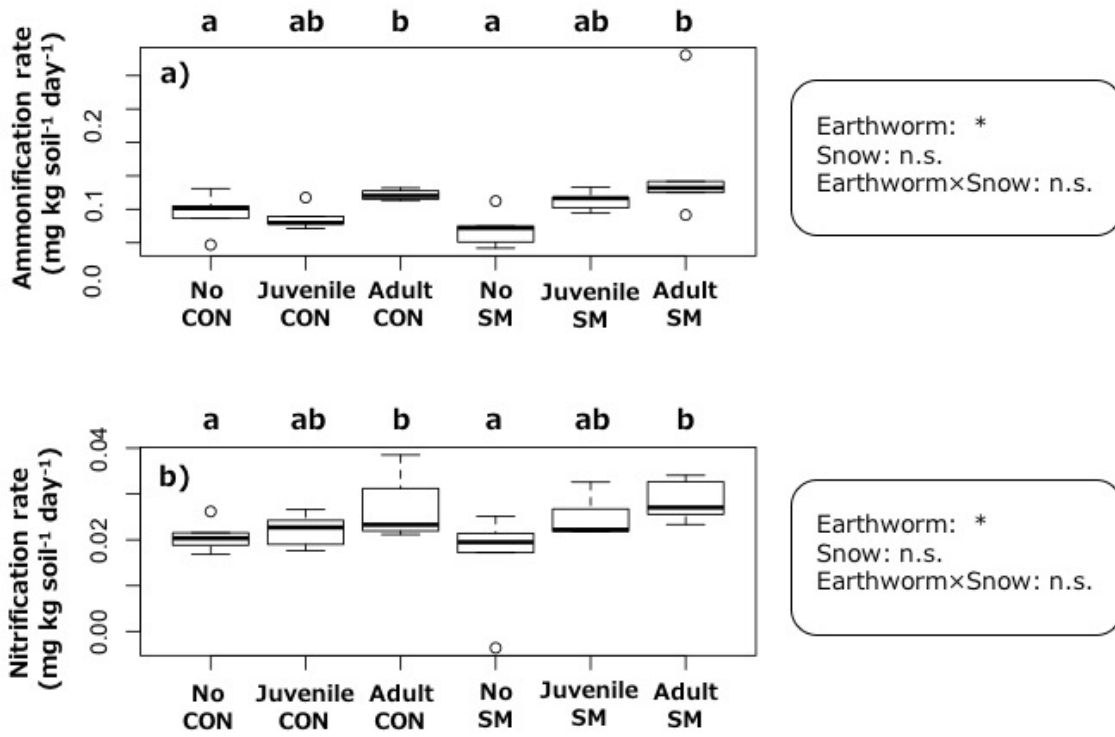
582 Fig. 4



583  
584  
585



586 Fig. 5



587

588