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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⁻²) is

150 within the range of the field observation (0-216 individuals m⁻²) which we conducted in

151 our experimental forests. The average fresh body weight of the adult individuals was
152 0.80 ± 0.18 g, and that of the juvenile individuals was 0.24 ± 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 27th November 2016, when the continuous snow cover starts in
161 northern Hokkaido, to 23rd 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 31st 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 31st March. In the snow-
173 removed plots, the snow disappeared on 7th April, while the snow in the control plots
174 disappeared on 21st 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 (23rd 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 NH_4^+ -N production (ammonification) and 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 NH_4^+ and 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 25th 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 23rd 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 NH_4^+ and
231 NO_3^- concentration between 27th November and 25th April and between 25th April and
232 23rd 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 ¹⁵N-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 2nd 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 1st
533 March to 31st 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

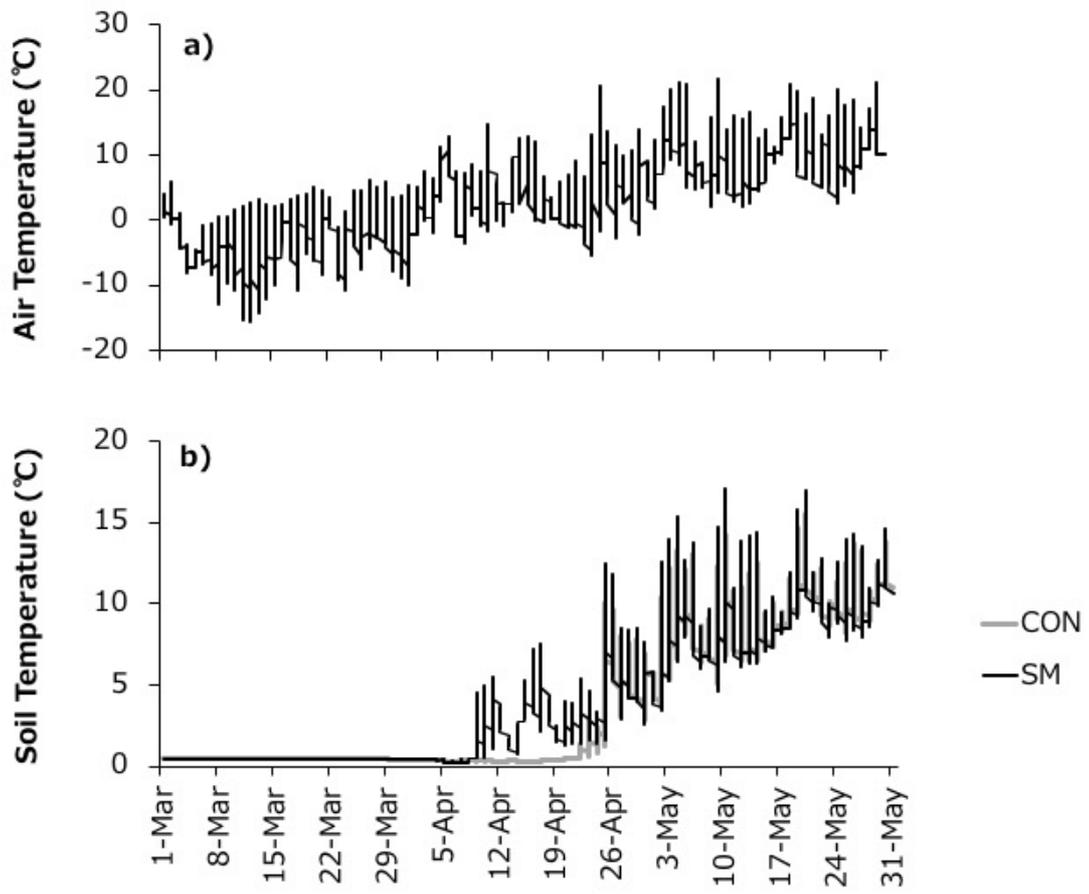
555 **Fig. 4.** The effect of snow reduction and earthworm treatment on ammonification rate
556 (a) and nitrification rate (b) in early spring. No = without earthworm, Juvenile = with
557 juvenile earthworm, Adult = with adult earthworm, CON = without snow manipulation
558 (control), SM = with snow manipulation. 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

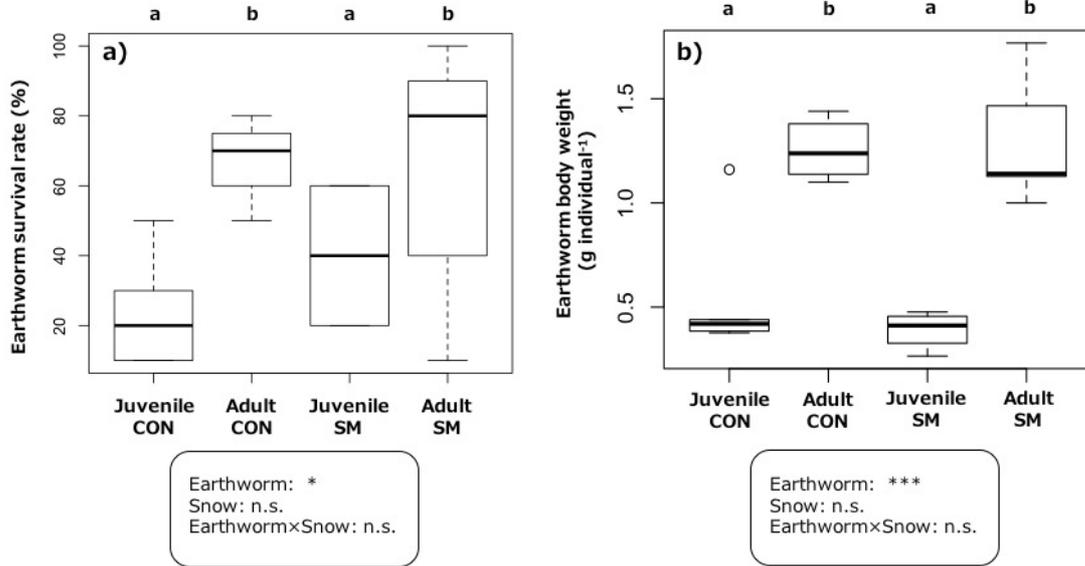
573 Fig.1



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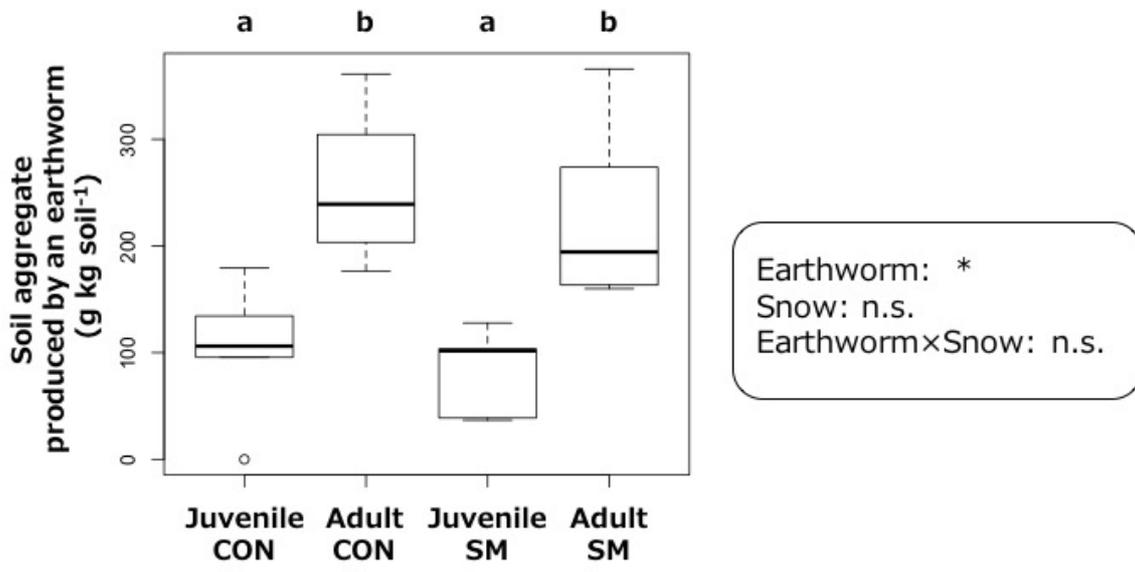
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576 Fig.2



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578 Fig. 3

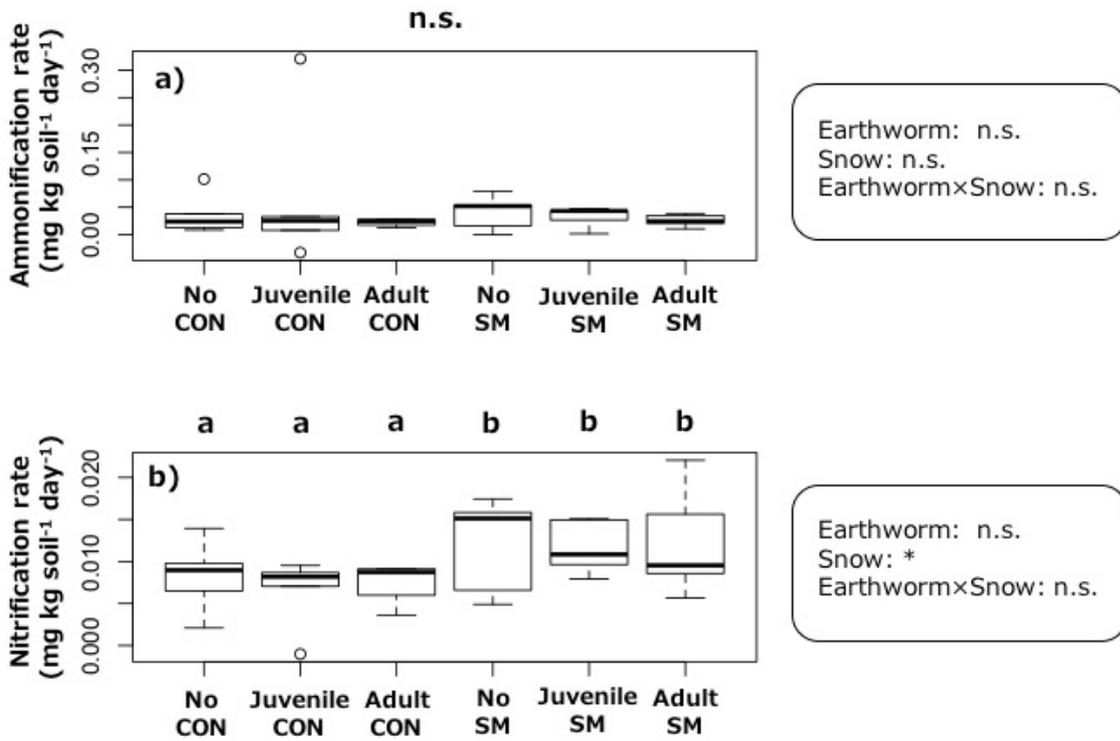


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582 Fig. 4

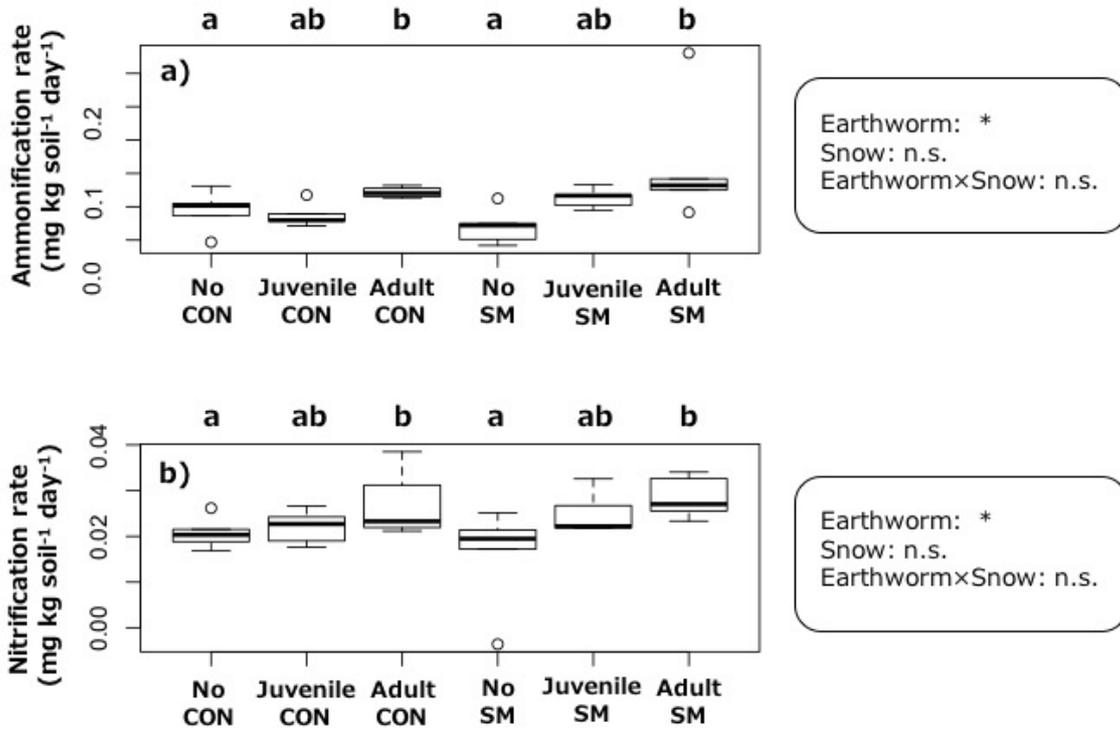


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586 Fig. 5



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