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**The effect of snow reduction and *Eisenia japonica* earthworm traits on soil
nitrogen dynamics in spring in a cool-temperate forest**

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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;

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

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

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

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

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

2. Materials and methods

2.1. Site description

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

(Makoto et al., 2014) and is predicted to decrease and advance in northern Japan
(Supplementary Fig. S1, Sapporo District Meteorological Observatory 2017).

2.2. Study design

We established 10 plots in Teshio Experimental Forest. All plots were apart from each other by five meters. In each plot, three mesocosm were placed. Each mesocosm was 30 cm long, 30 cm wide and 20 cm deep. The mesocosm sidewalls were made of polyvinyl chloride plates, and the upper and lower parts were made of nylon mesh (mesh opening: 2 mm). In the preliminary study, this mesh opening was sufficient to prevent the juvenile and adult *E. japonica*, whose size was used in this study, to escape from mesocosms. Each mesocosm contained 2.3 kg of wet soil (gravimetric moisture content was about 31%) after sieving (with a 4.75 mm sieve) to homogenize and remove, fine roots, gravel, and coarse woody debris were placed in each plot (a total of 30 mesocosms). In each plot, one of the three mesocosms contained 10 adult *E. japonica*, one contained 10 juvenile *E. japonica*, and the other contained no *E. japonica*. *E. japonica* is the most dominant geophagous earthworm species in northern Hokkaido (Nakamura, 1972). The density of the earthworm in our study (111 individuals m⁻²) is within the range of the field observation (0-216 individuals m⁻²) which we conducted in

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

2.3. Field manipulation

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

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

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

2.4. Earthworms

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

2.5. *Aggregate production*

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

2.6. *Soil nitrogen*

The net rates of NH_4^+ -N production (ammonification) and NO_3 -N production (nitrification) were calculated as the net changes in the soil pool for each ion during the incubation. Before and after the half-year incubation, eight grams of soil from each

mesocosm was shaken with 40 ml of 2 M KCl solution for one hour and filtered (Advantec 5B, Advantec Co Ltd., Tokyo, Japan). The concentrations of NH_4^+ and NO_3^- were determined using a continuous flow injection analyzer (AACS-4, BL-Tech Co. Ltd., Osaka, Japan). To check the effect of snow removal, we obtained the samples on 25th April, soon after snow disappearance when the effect of snow manipulation on surface soil is known to be profound (Blakinship et al., 2014), and on 23rd May, when the plants in forest ecosystems starts to increase their activity after snow disappearance. We measured the ammonification and nitrification rates as the net change in NH_4^+ and NO_3^- concentration between 27th November and 25th April and between 25th April and 23rd May 2017 as the value in early spring (soon after snow disappearance) and in late spring, one month after snow disappearance, respectively. Ideally, to check the influence of snow removal. we should have sampled the soil also just before the snow removal (end of March) to confirm the soil condition are similar across the mesocosms before the snow removal. However, not all snow can be removed to avoid the effect of direct disturbance of snow removal by shoveling on soil (Templer et al., 2012) and thus we assumed that there was no significant difference among the mesocosms before the snow removal, which we should be careful to interpret the data.

2.7. Statistical analysis

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

3. Results

3.1. Earthworm survival

The snow removal did not influence the survival rate of *E. japonica* (Fig. 2a). The 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)

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

4. Discussion

Contrary to our expectations, our results indicate that the importance of the earthworm presence was small to understand the consequence of snow removal on soil N dynamics from winter to early spring (Fig. 4). On the other hand, in a previous study, the milder winter is implicated to activate the earthworm in the temperate forests of US (Eisenhauer et al., 2014). However, no influence of snow reduction on the survival rate, body weight and aggregate production remonstrate that reduction of snow and earlier snowmelt does not likely to activate the earthworm in Japanese temperate forests. For the microarthropods, snow reduction in the beginning of winter was found to decrease the abundance of soil arthropods due to the increase in soil frost in cool-temperate forests (Templer et al., 2012). In our study, however, the snow reduction resulted in the early increase of the soil temperature and did not cause any soil frost (Fig. 1). In the 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

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

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

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

5. Conclusion

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

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389

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Legends to figures

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

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

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

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

Fig. 4. The effect of snow reduction and earthworm treatment on ammonification rate (a) and nitrification rate (b) in early spring. No = without earthworm, Juvenile = with juvenile earthworm, Adult = with adult earthworm, CON = without snow manipulation (control), SM = with snow manipulation. Different letters mean the presence of statistically significant difference analyzed with ANOVA with Tukey HSD test ($p < 0.05$). n.s. means the absence of statistically significant influence of the treatment. The asterisks show the level of significance by two-way ANOVA: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. n.s. indicates the lack of significant difference among the treatments.

Fig. 5. The effect of snow reduction and earthworm treatment on ammonification rate (a) and nitrification rate (b) in late spring. No = without earthworm, Juvenile = with 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.
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573 Fig.1

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578 **Fig. 3**

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