



# OPEN Responses of grassland soil mesofauna to induced climate change

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Climate change can significantly affect the below and above-ground ecosystems. This study aimed to test the effects of induced climate change on the composition of soil mesofauna and vascular plant species in semi-natural grasslands. Open-top chambers (OTCs) were used to manipulate climatic conditions. The research was carried out over three years in two semi-natural grasslands in south-west Poland (Central Europe). Changes in soil mesofauna (Collembola and Acari) and vegetation characteristics under OTC treatment were evaluated and compared to untreated control sites. Treatment with OTC significantly increased the abundance of Oribatida mites (up to 42%) but decreased the abundance of Gamasida (by 21%), indicating contrasting responses of the Acari subgroups to warming. Collembola diversity was significantly reduced under OTC conditions, as reflected in the lower Margalef, Simpson, and Shannon–Wiener indices. Furthermore, the abundance of epigeic Collembola increased under OTC. Redundancy analysis (RDA) revealed that plant traits explained 37.91% of the variation in mesofauna structure. Structural Equation Modelling (SEM) further supported these findings, showing that climate exerted a strong negative effect on soil quality, which in turn had a pronounced positive influence on plant quality (total effect = 0.678). Plant quality significantly enhanced soil fauna abundance (total effect = 0.264), while the overall impact of climate on soil fauna was negative (−0.231), primarily via indirect pathways. These findings suggest that climate change in grassland ecosystems can disrupt the ecological balance of soil fauna by modifying their responses to environmental variables. The SEM results emphasise the cascading nature of these effects, from climate to soil, vegetation, and ultimately soil fauna, highlighting the importance of indirect environmental pressures. Conserving plant diversity remains essential to buffer against climate-driven disruptions and maintain ecosystem stability.

**Keywords** Soil fauna, Plant diversity, Soil arthropods, Collembola open-top chamber (OTC), Structural equation modelling (SEM)

Extensively used grasslands are important in storing carbon dioxide due to the high stability of aboveground plant biomass<sup>1</sup>. It is also assumed that semi-natural grassland ecosystems can minimize the negative impact of climate change on belowground ecosystems<sup>2</sup>. The ability of grassland ecosystems to recover or adapt to climate-induced changes is a crucial consideration. Resilient ecosystems can better withstand disturbances and maintain their structure and function<sup>3</sup>. However, according to Schirpke et al.<sup>4</sup>, semi-natural grasslands from highland regions of the temperate hemisphere are highly vulnerable to climate change due to their sensitivity to precipitation, temperature, and CO<sub>2</sub> levels. The resilience of these ecosystems is also affected by excessive utilisation by grazing and/or mowing<sup>5</sup>. The impact of climate change on grassland ecosystems is not straightforward and depends on various circumstances.

Factors related to climatic changes can lead to changes in the vegetation structure of semi-natural grasslands, including reduced productivity and consequently negative impacts on animal feed consumption and reproduction<sup>6</sup>. Warming, summer drought, and CO<sub>2</sub> enrichment can also affect flowering phenology and plant community structure, affecting ecosystem functioning<sup>7</sup>. These vegetation-level responses indirectly influence soil organisms by altering the quality, quantity, and timing of soil litter and root exudates entering the

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soil. Therefore, vegetation changes serve as a key mediator of soil fauna dynamics under climate change<sup>8</sup>. The complex responses of semi-natural grasslands to climate and management changes highlight the need for more research in this area<sup>9</sup>.

Soil fauna, including macrofauna and mesofauna, play a crucial role in ecosystem functioning, particularly in nutrient cycling and decomposition<sup>10,11</sup>. They interact with the diversity of plant litter species, influencing decomposition rates and thus carbon and nutrients<sup>10</sup>. The most numerous representatives of soil fauna are Collembola and Acari in grassland ecosystems<sup>12,13</sup>, being important bioindicators of the biological quality of the soil, so changes in their occurrence can indicate possible negative or positive changes in the ecosystem<sup>14,15</sup>. The responses of soil biota to global change, including elevated CO<sub>2</sub>, warming, and altered precipitation, are complex and can vary based on factors such as taxonomy, body size, and feeding habits<sup>16</sup>.

Numerous studies have found a significant impact (mostly negative) of climate change on the occurrence of soil fauna<sup>17–19</sup>. Importantly, these effects often occur through indirect pathways, such as changes in plant biomass and litter input, changes in root dynamics, and soil microclimate alterations induced by vegetation structure<sup>20,21</sup>. However, such generalisations might overlook site-specific indirect effects. Kardol et al.<sup>22</sup> in the field experiment in a subtropical climate (USA) demonstrated that changes in soil moisture content, influenced by precipitation and temperature, impact soil microarthropod communities (mainly Acari and Collembola) more than the direct effects of warming and elevated CO<sub>2</sub>. Climate change, particularly in the form of increased temperature and dryness in microcosm experiments, has been shown to have significant impacts on soil food webs (predatory mites - Collembola), with potential cascading effects on higher trophic levels<sup>6</sup>. A study by Yin et al.<sup>23</sup> demonstrated that climate change and land use practices significantly affect the density of different forms of Collembola life. Surface-dwelling species, especially epigeic (epigeic) collembolans, are particularly vulnerable to environmental changes, underscoring the need for targeted conservation efforts and further research on their resilience. These large-bodied epigeic Collembola exhibit high metabolic activity, consume high-quality food substrates, and primarily inhabit the surface. In contrast, euedaphic species are small, deep-dwelling organisms with a low metabolic activity that feed on lower-quality food. They are characterised by their colorlessness and reduced appendages, such as the furca, antennae, and legs. Hemiedaphic species (mainly litter dwelling) possess intermediate traits between the epigeic and euedaphic groups<sup>24</sup>. Xu et al.<sup>25</sup> observed a reduction in Collembola richness and biomass in response to seasonal drought and air warming, with the effects varying by soil type. Jucevica and Meleciš<sup>26,27</sup> reported a decrease in Collembola species richness and changes in community structure in response to long-term climate warming, and the latter study highlighted the role of temperature and precipitation in driving these changes.

According to mites, the responses to warming were inconclusive. Acari biomass (particularly Oribatida mites) significantly deduced in warmer soils<sup>28</sup>. In the subarctic ecosystem, the warming treatment significantly decreased the number of Oribatida and Gamasida mites<sup>29</sup>. In a long-term warming experiment in the tundra ecosystem, warming negatively only affects the juvenile stages of Oribatida, with no effect on adult mites or annual abundance<sup>30</sup>. Based on previous studies, we can assume that climate change can have significant direct and indirect impacts on Collembola and more ambiguous effects on Acari. In particular, vegetation-mediated effects, such as altered litter input, shading, root turnover, and moisture retention, play a central role in shaping soil fauna responses and should be emphasised in future research frameworks.

This study examines the effects of induced climate change on soil mesofauna and vegetation in semi-natural grasslands. To achieve this, open-top chambers (OTCs) were utilised, which passively increased the air temperature and reduced the amount of precipitation<sup>31</sup>.

We hypothesised that induced changes in climatic conditions will:

1. Alter soil mesofauna community structure, with warming and reduced precipitation leading to decreased diversity and abundance, particularly among moisture-sensitive taxa.
2. Affect Collembola lifeform groups differentially, with surface-dwelling (epigeic) species expected to increase due to higher availability of fresh litter, while deep-dwelling (euedaphic) species decline due to reduced soil moisture and altered microhabitats.
3. Cause vegetation-mediated shifts in mesofauna, where changes in plant nutrient content, biomass, and litter input—driven by climate manipulation—will significantly explain variation in mesofauna abundance and diversity.

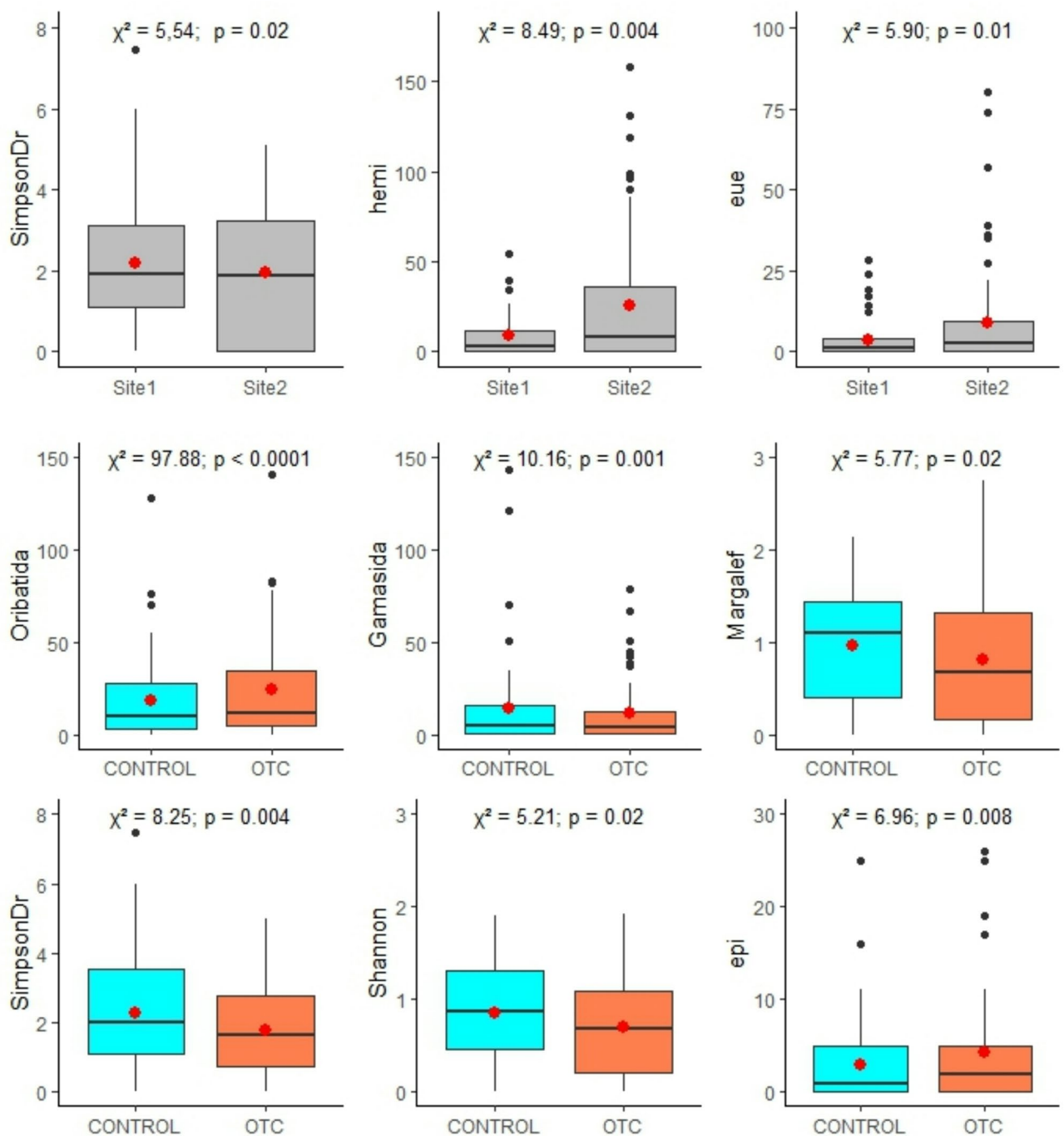
## Results

### Soil fauna responds to climate change

The effects of induced climate change on various groups of soil organisms were analysed, with a focus on Collembola abundance, diversity and ecomorphological classifications (epigeic, hemiedaphic, and euedaphic), as well as Acari abundance, particularly within the Oribatida and Gamasida groups. Treatment effects (OTC versus Control) significantly influenced Oribatida (42% increase under OTC,  $p < 0.0001$ ) and Gamasida abundance (21% decrease under OTC,  $p = 0.001$ ), as well as Collembola diversity, as indicated by Margalef indices (16% decrease under OTC,  $p = 0.016$ ), Simpson's dominance indices (21% decrease,  $p = 0.005$ ) and Shannon-Wiener indices (18% decrease,  $p = 0.022$ ). A significant impact of the treatment was also observed for epigeic forms (42% increase under OTC,  $p = 0.008$ ) (Fig. 1, Fig. S5).

The effect of the site significantly affected the Simpson Dr diversity index of Collembola ( $p = 0.021$ ) and hemiedaphic Collembola ( $p = 0.011$ ) and euedaphic Collembola ( $p = 0.01$ ) (Fig. 1, Fig. S5), with a higher organism number and diversity of organisms at Site 2.

A significant interaction was observed between site and treatment for the abundance of Collembola and Oribatida, Simpson's diversity index, and the three morphological groups of Collembola: epigeic, hemiedaphic and euedaphic. Specifically, the total abundance of Collembola decreased by 26% in the OTC treatment at



**Fig. 1.** The effect of Site (Site 1 ~ Site 2); treatment (OTC ~ Control) and the interaction between site and treatment on different soil faunal groups and their diversity measures. Oribatida, Gamasida—mites groups; Simpson Dr, Shannon, Margalef—indices calculated based on Collembola taxa diversity; Collembola—Collembola community abundance; epi—epigeic Collembola abundance; hemi—hemiedaphic Collembola abundance; eue—euedaphic Collembola abundance.

Site 2 ( $p < 0.0001$ ). Within this group, the euedaphic Collembola showed a 41% decrease ( $p = 0.07$ ), while the hemiedaphic Collembola decreased by 30% at the same site. Epigeic Collembola exhibited a 59% increase in abundance under OTC treatment at Site 2 ( $p < 0.0001$ ). For Oribatida, the abundance increased with OTC treatment by 10% at Site 2 and by 27% at Site 1 ( $p < 0.0001$ ). Simpson's diversity index decreased 46% in OTC plots at Site 1 (Fig. 1, Fig. S5).

#### Responses of soil fauna combined with vegetation indices

RDA analysis showed 37.91% of the variation explained (Fig. 2; Tables 1 and 2). The explained Axis 1 was strongly linked to the response variables (including Gamasida and Collembola) and plant traits. Axis 2 was related to

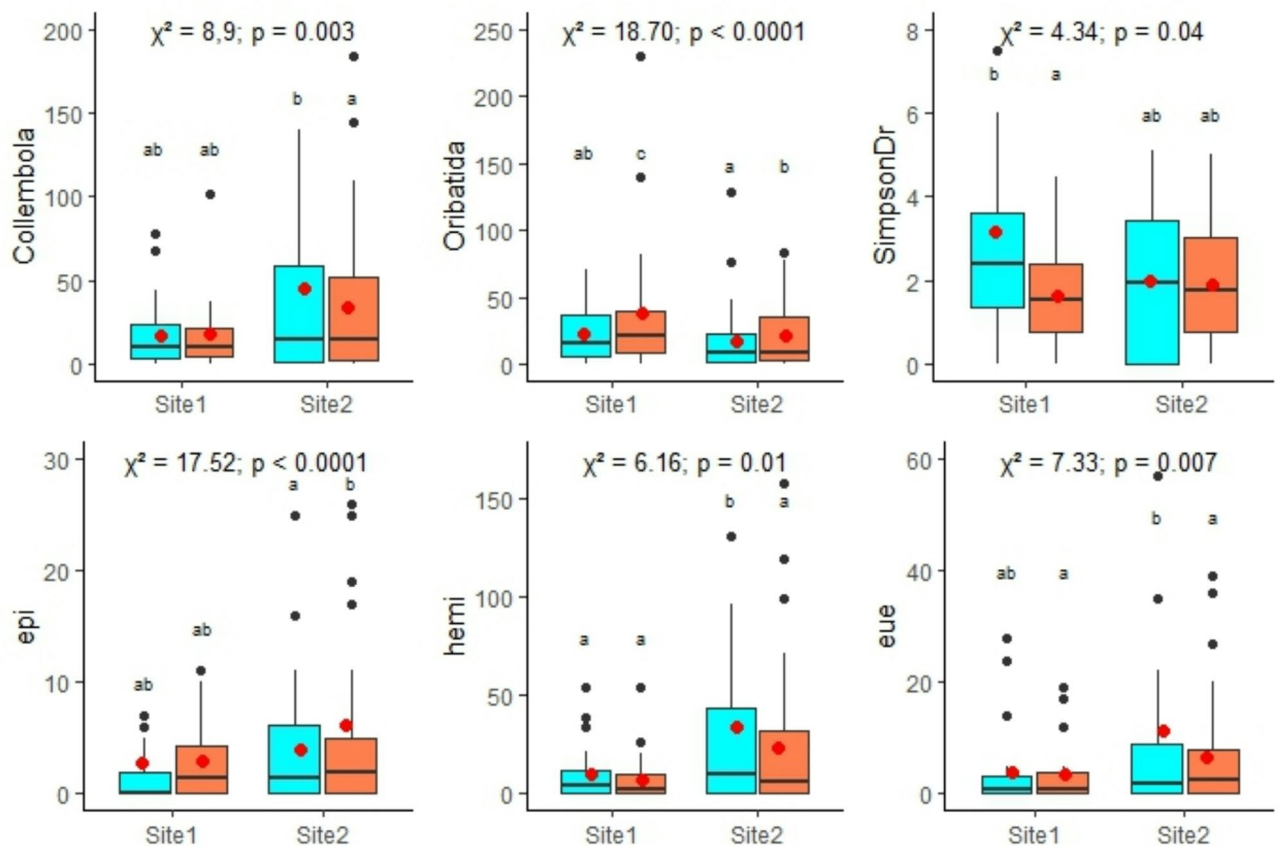


Figure 1. (continued)

Oribatida mites and soil characteristics. There is also a significant difference between habitat preferences (soil and vegetation characteristics) between Site 1 and Site 2, and smaller differences between OTC and control. The factors that contributed most to the variance explained were plant traits: P, Mg, dry mass, and N. From the characteristic traits, the main contribution was related to pH.

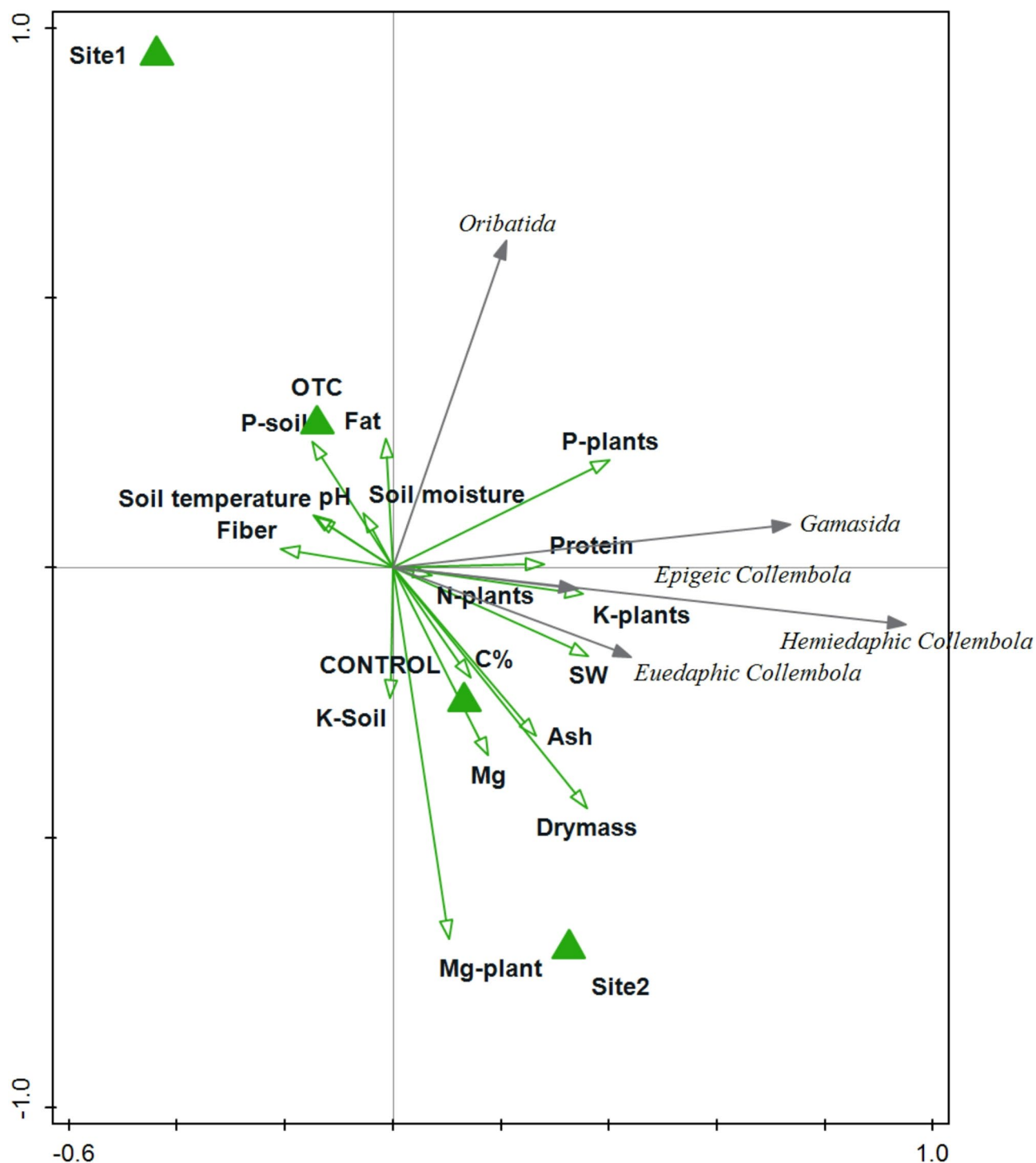
To evaluate the effects of environmental and plant-related variables on soil fauna, generalised linear models (GLMs) were applied separately to Oribatida, Gamasida, epigeic Collembola, hemiedaphic Collembola and euedaphic Collembola (Table S2). All models were statistically significant, indicating that the selected predictors were relevant for each group. Protein content in the plants had a significant positive effect on all faunal groups analysed, showing a consistent response pattern in taxa. The nitrogen content in plants showed a significant negative impact on Gamasida, hemiedaphic Collembola, euedaphic Collembola, and Oribatida. The effect was not significant for Epigeic Collembola. The soil moisture had a significant negative impact on Gamasida, epigeic Collembola, hemiedaphic Collembola, and euedaphic Collembola. It was not significant for Oribatida. Soil pH showed a significant negative impact across all faunal groups.

Furthermore, the structural model revealed several significant relationships (Fig. 3). Climate had a strong negative effect on soil quality, while both climate and soil quality exerted significant positive influences on plant quality. In turn, plant quality positively affected soil fauna, whereas climate had a marginally significant negative effect on soil fauna. The analyses of indirect effects further clarified these dynamics. Although the climate showed a direct positive effect on plant quality (0.188), its total effect was slightly negative (− 0.087) due to a strong negative indirect pathway through soil quality (− 0.276). Similarly, the total effect of climate on soil fauna was negative (− 0.231), combining a moderate direct effect (− 0.173) and a weaker indirect effect (− 0.058), mediated primarily by changes in soil and plant quality. The strongest positive total effect in the model was from soil to plant quality (0.678). The soil also influenced the soil fauna, with a total effect of 0.264, mainly through an indirect pathway through the quality of the plants (indirect effect = 0.178). Overall, the model highlights a cascading sequence of interactions: Climate → Soil Quality → Plant Quality → Soil Fauna, highlighting that biotic responses are predominantly shaped by indirect environmental pressures (Table 3).

## Discussion

### The general effect of OTC on temperature and soil moisture-pros and cons of the method.

The crucial factors in climate change include increased CO<sub>2</sub> concentration, altered precipitation patterns, extreme climatic events, and temperature change<sup>32</sup>. In our experiments, we observed the effect of increased air temperature, which range (ca. 1 °C) is slightly less compared to projected in the near future (2021–2050) in Poland, for the RCP 8.5 scenario assessed as +1.3 °C<sup>33</sup>. Because the construction of OTC did not cause



**Fig. 2.** The RDA biplot showing the soil fauna responses to the plant traits, site characteristics (Site 1 ~ Site 2), and climate effects (Control ~ OTC) as explanatory variables.

significant soil moisture, thus we did not observe the effect of precipitation. Additionally, we did not influence or measure changes in CO<sub>2</sub> levels.

### Mesofauna

Our study suggests that Open-Top Chambers (OTCs) significantly increased the abundance of Oribatida mites, indicating that these mites may respond positively to certain environmental changes. This increase is likely associated with the microclimatic modifications induced by the chambers, such as elevated temperatures. The increase in Oribatida abundance is consistent with previous research showing that the abundances of these

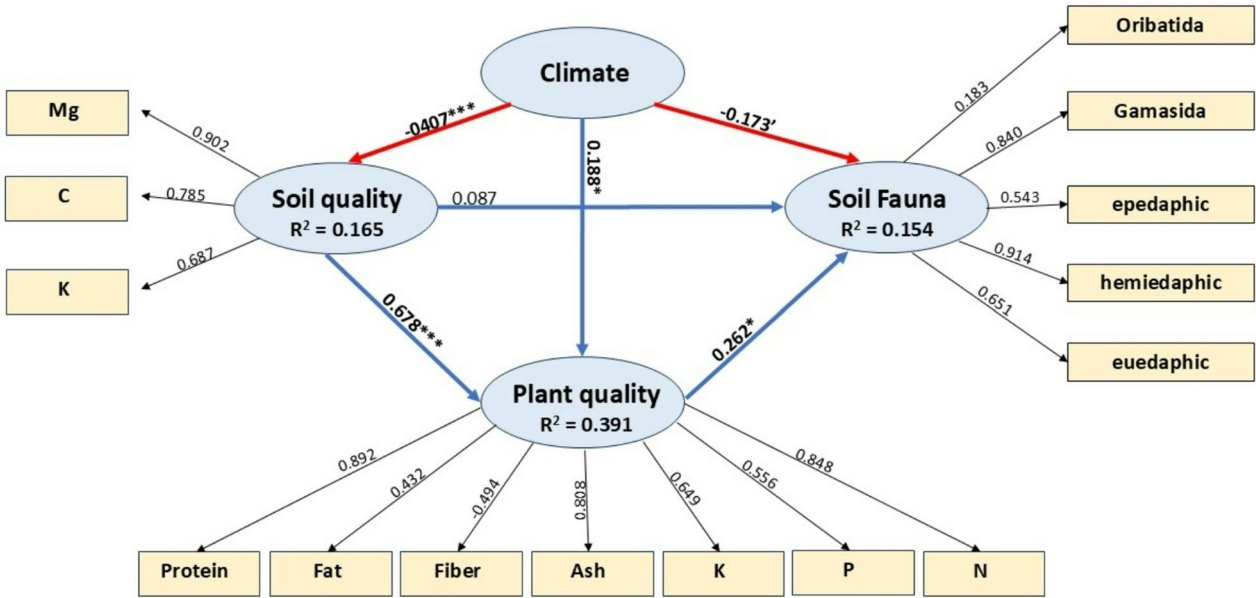


Statistic	Axis 1	Axis 2
Explained variation (cumulative)	0.361	0.083
Explained by constrained axis (%)	36.10	44.39
Pseudo-F value	0.8208	0.6444
P value	0.001	0.001
Adjusted explained variation	37.91%	

**Table 1.** Summary statistics of axis variation, significance of RDA, and results of testing individual constrained axes.

Faktor	Explains %	Contribution %	Pseudo-F	P
P-plants	6.58	13.46	7.5	0.001
Mg-plants	8.80	17.98	10.9	0.001
Dry mass-plants	2.79	5.71	3.5	0.024
N-plants	13.46	27.51	22.3	0.001
Fat-plants	1.98	4.05	3.4	0.019
Fiber-plants	1.51	3.09	2.0	0.076
Ash-plants	1.61	3.30	2.7	0.028
pH-soil	1.89	3.86	2.4	0.065
P-plants	6.58	13.46	7.5	0.001
Mg-plants	8.80	17.98	10.9	0.001

**Table 2.** Variation explained by environmental variables with significant distribution.



**Fig. 3.** The partial least squares structural equation model (PLS-SEM) shows the effects of climate, soil quality, and plant quality on soil mesofauna. Numbers adjacent to arrows (red and blue) are standardized path coefficients. The blue line represents a positive correlation, while the red line represents a negative correlation. Numbers adjacent to black arrows are outer loadings. R is the proportion of variance explained. Significance levels: \* indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$  and \*\*\* indicates  $P < 0.001$ , ' indicates marginal effect (close to  $P = 0.05$ ).

mites increase at elevated temperatures, benefiting from their role in the decomposition of organic material<sup>16,29</sup>. However, there is limited data on this phenomenon in temperate grasslands. Oribatida are crucial in ecosystem functions, particularly in nutrient cycling and organic matter breakdown. Therefore, the growth of their population under OTC conditions could have broader ecological implications<sup>34</sup>. Gamasida mites play a role

Relation	Direct	Indirect	Total
Climate → Soil quality	− 0.4067	0.0000	− 0.4067
Climate → Plant quality	0.1883	− 0.2756	− 0.0874
Climate → Soil Fauna	− 0.1733	− 0.0582	− 0.2315
Soil quality → Plant quality	0.6778	0.0000	0.6778
Soil quality → Soil Fauna	0.0868	0.1776	0.2644
Plant quality → Soil_Fauna	0.2620	0.0000	0.2620

**Table 3.** Valuation of the structural model showing direct, indirect and total effects.

as free-living soil-dwelling, litter, or soil surface predators. For this group of mites, the negative impact of OTC was observed, which is consistent with the results of other experiments. In two different peatland experiments, the Gamasida abundance decreased with warming<sup>29,35</sup>. Gamasida mites were also observed to depend on microclimatic conditions, including soil moisture and temperature under forest conditions<sup>36</sup>.

We also found that induced climate change significantly decreased the diversity of Collembola, which supports our first hypothesis. One possible explanation is that the OTC chambers create a more homogeneous environment, favouring a few dominant species that thrive under these modified conditions while outcompeting less competitive species<sup>37</sup>. Alternatively, changes in microhabitat caused by OTCs, such as alterations in soil structure or reduced leaf litter, may limit the available niches for diverse Collembola species<sup>38</sup>. These changes could disproportionately affect species with specific habitat requirements or specialised diets, decreasing species richness<sup>39</sup>. Similar trends of reduced functional diversity related to microclimate and habitat structural complexity have been observed in Collembola communities in geothermal warming sites in Iceland<sup>40</sup>. However, there is a lack of evidence in the literature regarding similar decreases in species diversity within temperate grassland ecosystems.

We cannot confirm our second hypothesis, which suggested that induced climate changes would have varying effects on the three Collembola lifeform groups: epigeic, litter-dwelling (hemiedaphic) and soil-dwelling (euedaphic)—due to their specific adaptations. Although we observed changes in overall Collembola diversity and abundance, the expected differential responses among these life-form groups were not apparent. This may indicate that while climate changes affect Collembola populations, the specific adaptations of these groups do not result in the anticipated variations in their responses. In contrast, other studies on global warming, such as that by Yin et al.<sup>23</sup>, have shown that Collembola life-form groups can react differently to climate change.

**Vegetation characteristic reaction during climate changes**

Generally, mountain and highland grasslands are considered liable for climate change: the former being limited by low temperature, the latter by drought and heat<sup>41,42</sup>. The initial physiological and metabolic responses to climate changes are fast, while significant changes in the structure and composition, such as species reordering and species gain and loss, are relatively slow processes<sup>43</sup>. After three years of our experiment, we show that climate warming does not support biodiversity (lower species richness and Shannon-Wiener index in the OTC), as suggested by trends observed in the RDA analysis. Furthermore, according to RDA results, climate warming is also not conducive to higher biomass production. According to Liu et al.<sup>44</sup>, the results of manipulative experiments in alpine grasslands using open-top chambers or heaters are rather inconsistent, revealing both an increase and a decrease in biomass production. Overall, the results differ with respect to drought manipulation, temperature warming, and a combination of both. This suggests that the process is complex and depends on many factors. To maintain the ecological and economic values of grasslands under future climate changes, in addition to the change in grassland productivity, we need to understand the effects of climate on forage quality<sup>44</sup>. Our experiment shows that the crude fat and fiber are higher in biomass in OTC stands (warmer climate); in turn, the biomass from control stands contains more magnesium. The changes in chemical composition and digestibility could emerge from both: a shift in species composition as well as a shift in the phenological stage<sup>41</sup>. Unfortunately, monitoring forage quality in semi-natural species-rich grasslands is rarely done due to the inherent complexity in determining forage quality and high variability within natural systems. As a result, the effects of climate change from warmer and drier conditions are strongly site dependent and highly variable across forage quality parameters<sup>44</sup>.

**Changes in soil-faunal communities driven by vegetation characteristics**

In these studies, soil fauna groups, such as Oribatida, Gamasida, and Collembola, responded differently to environmental variables, including plant biomass, nitrogen (N), phosphorus (P), and magnesium (Mg). The contribution of soil characteristics to the variation explained by mesofauna responses was lower. This highlights the critical role of plant traits and nutrient availability in shaping soil biodiversity.

Research indicates that Open Top Chambers (OTCs), designed to simulate warming, alter the relationship between environmental factors and soil mesofauna. Thakur et al.<sup>45</sup> reported that climate-induced changes could affect soil detritivore activity, with cascading impacts on decomposition and nutrient cycling. While control treatments promote the diversity and richness, OTCs negatively impact vegetation. Eisenhauer et al.<sup>46</sup> highlighted the importance of plant species richness as a primary driver of soil community dynamics, while Scherber et al.<sup>47</sup> emphasized its positive impact on multiple trophic levels. These findings suggest that warming and altered climatic conditions, as simulated by OTC, can disrupt the ecological balance of soil fauna by modifying their responses to environmental variables, including plant biomass and nutrient availability. Additionally, the content

of plant protein emerged as a significant positive factor influencing the abundance and diversity of all faunal groups analysed, showing a consistently positive response between different taxa. This suggests that plant protein significantly contributes to food quality or nutrient availability for these organisms, which was not previously described in the literature. In contrast, nitrogen content in plants exhibited a significant negative effect on several soil fauna groups, including Gamasida, hemiedaphic Collembola, euedaphic Collembola, and Oribatida, while the effect was not significant for epigeic Collembola. Elevated nitrogen levels in plants could lead to changes in food quality, making it less favourable for certain soil organisms<sup>48</sup>. Epigeic Collembola was less sensitive to changes in nitrogen levels, possibly due to its ability to exploit a wider range of organic matter or a more opportunistic feeding strategy<sup>34</sup>. Soil moisture also had a significant negative impact on multiple faunal groups, including Gamasida, epigeic Collembola, hemiedaphic Collembola, and euedaphic Collembola. The reduction in soil moisture can decrease the activity and survival rates of soil fauna, especially those that rely on consistent moisture levels for respiration, feeding, and reproduction<sup>49</sup>. The soil pH showed a significant negative effect in all faunal groups, indicating that increased soil acidity can be detrimental to soil fauna. Acidic soils may lead to unfavourable changes in fauna, communities, and the physical properties of the soil, affecting the availability of nutrients and the general suitability of the habitat of soil organisms<sup>50</sup>, particularly if at certain sites of the experiment, pH was below 4. Structural Equation Modelling (SEM) provided further insight into the complexity of these relationships by identifying direct and indirect pathways through which climate influences soil fauna. The analysis revealed that climate primarily affected soil fauna indirectly by altering soil quality and, in turn, vegetation characteristics, which was confirmed by other studies<sup>51</sup>. Plant quality emerged as a key mediator, linking changes in soil conditions with faunal responses, which is consistent with the literature<sup>51</sup>. These findings suggest that indirect effects, especially those mediated through vegetation, play a dominant role in shaping the belowground ecological responses to warming. SEM also highlighted a cascading sequence of interactions—from climate to soil, to vegetation, and ultimately to soil fauna—illustrating the interconnected nature of these ecological systems.

To conclude, our study demonstrates that Open Top Chambers (OTCs) effectively simulate warming, increasing air temperature by ~1 °C without altering soil moisture. OTCs influenced soil mesofauna differently: Oribatida abundance increased due to warming-enhanced activity, while Gamasida declined, probably due to their sensitivity to microclimatic changes. Collembola diversity decreased as warming created homogeneous conditions that favour dominant species. The changes in vegetation traits and soil characteristics mediated the changes in soil fauna characteristics. SEM analysis reinforced these patterns by confirming the importance of indirect pathways, particularly through changes in vegetation, as the primary drivers of soil faunal dynamics under warming conditions. Vegetation in OTC plots showed reduced biodiversity and species richness, with biomass exhibiting higher crude fat and fiber but lower magnesium than controls. These findings align with previous studies and highlight the complex, site-dependent effects of climate warming on soil fauna, plant communities, and forage quality. Conserving plant diversity remains essential to buffer against climate-driven disruptions and maintain ecosystem stability.

## Methodology

### Study sites

The experiments were conducted in two sites in the South–West Part of Poland: the Stołowe Mountains - National Park (Site 1) and the Kaczawskie Mountains-Landscape Park (Site 2). In Site 1, 8 experimental plots, and in Site 2, 12 experimental plots have been established (Fig. S1). The climate of both sites is a moderate mountain and submontane, with a mean temperature of 6.3 °C and a yearly sum of precipitation of 869 mm in Stołowe Mountains, and 7.7 °C and 769 mm, respectively, in Kaczawskie Mountains (*imgw.pl*). The soil conditions were characterized in Table S1.

According to the EUNIS Habitat Classification expert system<sup>52</sup>, in Site 1, hay meadows (habitat code-R23) occurred, while at Site 2—low and medium altitude hay meadows (habitat code-R22). A total of 62 vascular plant species were recorded in the permanent plots at Site 1, including 11 grass species, 44 forbs species, 5 species of sedges, and 2 species of trees in the seedling phase. The most frequently recorded was: *Deschampsia flexuosa* (L.) Trin., *Festuca rubra*, *Holcus mollis* L., *Hypericum maculatum* Crantz, *Agrostis capillaris* L., *Veronica chamaedrys* L., *Stellaria graminea* L., *Campanula patula* L., *Anthoxanthum odoratum*, and *Luzula multiflora* (Retz.) Lej. Most plots were located in vegetation dominated by *Deschampsia flexuosa* and *Festuca rubra* with a high proportion of forbs listed above. In Site 2, a total of 117 vascular plant species were recorded, including 20 grass species, 84 forbs species, 7 sedge species, and 6 tree species in the seedling phase. The most frequent were: *Dactylis glomerata* L., *Arrhenatherum elatius* L., *Agrostis capillaris* L., *Holcus lanatus* L., *Festuca rubra* agg. L., *Plantago lanceolata* L., *Anthoxanthum odoratum* L., *Galium mollugo* L., *Lathyrus pratensis* L., and *Veronica chamaedrys* L. The studied plots were placed in vegetation varying along the moisture gradient. The most xerothermic vegetation was dominated by *Festuca rubra*, *Anthoxanthum odoratum*, *Hieracium pilosella* L., *Thymus pulegioides* L., and *Dianthus deltoideus* L. Vegetation developed in the most nutrient-rich and moisture habitats was dominated by *Schedonorus pratensis* (Huds.) P. Beauv., *Arrhenatherum elatius*, *Festuca rubra*, and *Juncus effusus* L. The structure of vegetation in Site 1 and Site 2 is presented by the Nonmetric Multidimensional Scaling (NMDS) in the graph (Fig. S2).

### Experimental design

The experiment was established in 2021. Two paired sampling subplots were established on each plot: one subplot under the climate manipulation using Open Top Chamber (OTC), and the second one in the neighbourhood control area beside. The OTC was made of acrylic glass on a metal frame, with an uncovered upper site, according to the scheme of Welshofer et al.<sup>31</sup>. The diameter of each OTC was 2 m and the height was 1.5 m. The size of the uncovered area was 0.75 m<sup>2</sup>. The experimental plots were mowed once a year in autumn each year. The scheme



of the OTC is presented in Fig. S3. During the experiment, the soil moisture (at 6 cm below ground) and soil and air temperature (at 15 cm above, 0 cm, and 8 cm below the ground) were monitored every 15 min using a TMS-4 dataloggers<sup>53</sup> at each plot in the control and OTC area. To convert raw soil moisture measurements into volumetric soil water content (VWC in  $\text{m}^3/\text{m}^3$ ), we used the universal calibration equation for mineral soils<sup>54</sup> implemented in the R package *myClim*<sup>55</sup>. We then used the functions of *myClim* to calculate the mean annual temperature for each depth and VWC for the year 2022. Results showed that the OTC increased air temperature by an average ca. 1 °C but did not significantly change the soil moisture (Fig. S4). We observed different effects of OTC on soil temperature: no significant changes at Site 1 and a significant increase at Site 2.

## Soil fauna and vegetation sampling

### Soil fauna

Soil samples for soil fauna abundance were taken at the beginning of July in three seasons in the years 2022, 2023, and 2024, 1 year after the OTCs were installed (2021). Three soil subsamples were taken diagonally across the center part (1  $\text{m}^2$  from each experimental area (OTC and control) from a depth of 10 cm using a core sampler with a diameter of 5 cm. One aggregate sample for three subsamples is used for further analysis. Soil organisms were extracted in Berlese-Tullgren funnels for 48 h using sieves with a mesh size of 0.2 cm. The extracted organisms were kept in 75% alcohol. All invertebrates were determined to be at the most possibly specific level (Acari-suborders, Collembola-species or genus).

Additionally, Collembola was classified into three morphological groups: epigeic, hemiedaphic, and euedaphic, according to Yin et al.<sup>23</sup>. We used the Margalef index, Simpson Dr index, and Shannon-Wiener index to assess the diversity of Collembola based on the species of genus level. The Margalef index measures species richness in a community, considering the number of species and individuals. Higher values indicate greater richness. The value of the Simpson's Reciprocal Index ranges from 1 to S (the total number of species). A higher Simpson Dr indicates greater diversity, meaning the community has many species that are evenly distributed. The Shannon-Weaver Index ranges from 0 to  $\ln(S)/\ln(S)\ln(S)$ , where SSS is the number of species. Higher values indicate greater diversity, while lower values suggest less diversity.

### Vegetation analysis

For vegetation analyses, subplots sized 1 × 1 m were located on OTU (treatment) and in the close vicinity of OTU (control). In each subplot, in the third decade of June 2024, species coverage was determined using a percentage scale. Species composition was used to assess biodiversity metrics for all species and for graminoids and forbs separately. For diversity metrics, we used species richness, the Shannon-Wiener index, and the Pielou index. The nomenclature of vascular plant species was unified according to the Euro + Med PlantBase (Euro + Med, 2006). Subsequently, the vegetation from 1  $\text{m}^2$  was mowed at a height of 3 cm, the biomass was dried and weighed, and the chemical analysis of the hay was conducted; including crude ash, crude protein, crude fat, crude fiber, as well as nitrogen, potassium, phosphorus, magnesium and calcium contents.

## Statistical analysis

All the statistical analyses were done in the R statistical environment. The effects of the treatment, site, and interactions were analyzed with the fitting generalized linear mixed-effects models (glmer) using the “lme4” package<sup>56</sup>; the fixed effects were the treatment (Control and OTC), the site (Site1 and Site2), and their interactions, whereas the random effect was the id of paired plots. The significance of the interactions and main effects was assessed using type III analysis of variance in the “lmerTest” package. Post hoc pairwise comparisons were performed using Tukey's multiple comparison test, with Bonferroni correction, in the “emmeans” package. In the case of overparameterization, we applied the generalized linear mixed models using a template model builder (glmmTMB) in the “glmmTMB” package, which is recommended by Kruppa and Hotchorn<sup>57</sup>. The model's diagnostic was performed using the “DHARMA” package.

The effects of treatment, site, and interaction on the species composition were examined by permutational analysis of variance (PERMANOVA) on Hellinger-transformed species data using the “vegan” package. To control the effect of paired plots, we applied the restricted permutation by using the code of a particular pair as a strata, and post-hoc test was used by applying pairwise.adonis2 function.

Redundancy analysis (RDA) was performed to examine the variations in soil fauna communities in response to environmental factors including Shannon-Wiener index, crude protein, crude fat, crude fiber, crude ash, Mg, N, P, K, biomass), soil traits (mean monthly soil temperature and monthly soil moisture before soil sampling from the depth 8 cm, phosphorus (P), magnesium (Mg), carbon (C) content and pH), as well site characteristics (Site 1 ~ Site 2), and climate effects (Control ~ OTC). The collinearity of the environmental factors has been excluded using variance inflation factors. The analysis was conducted using CANOCO 5.0, with the response data transformed using the log function ( $\log(1 \cdot Y + 1)$ ). The influence of explanatory variables on community composition was evaluated, and the significance of individual constrained axes was tested.

Additionally, a generalized linear model (GLM) was employed to assess the impact of plant traits, soil parameters, site characteristics (Site 1 ~ Site 2), and climate effects (Control ~ OTC) on specific soil fauna groups. For each group, the best-fitting distribution was selected using the Akaike Information Criterion (AIC), with lower AIC values indicating a better model fit. While RDA provided an overall, multivariate view of the relationship between explanatory variables and soil fauna community structure, GLM was used for a more targeted analysis of how these variables influenced the abundance or presence of specific groups within the community. This combination of approaches allowed for a broad overview of the community dynamics and a more focused understanding of the effects on individual taxa.

To analyse the relationships among climate, soil quality, plant quality, and soil fauna, we used partial least squares structural equation modeling (PLS-SEM). The variables were grouped into four latent constructs:

climate (soil moisture), soil quality (magnesium, potassium, and organic carbon), plant quality (protein, fat, fiber, ash, K, P, N), soil fauna (Oribatida, Gamasida, Epigeic, Hemiedaphic, and Euedaphic). The structural model specified directional relationships: (a) climate influences soil quality, plant quality, and soil fauna; (b) soil quality influences plant quality and soil fauna; (c) plant quality influences soil fauna. The goodness of fit of the PLS-SEM was assessed using various criteria, including the Goodness-of-Fit (GoF) index, indicator internal consistency reliability (Cronbach's alpha [CA] > 0.6, Composite reliability [CR] > 0.6), convergent validity (Average variance extracted [AVE] > 0.5), coefficient of determination ( $R^2$ ,  $R^2_{\text{weak}} = 0.19$ ,  $R^2_{\text{moderate}} = 0.33$ ,  $R^2_{\text{substantial}} = 0.67$ )<sup>58</sup>. Most blocks demonstrated satisfactory internal consistency and convergent validity, with CR > 0.6, CA > 0.8, and AVE nearly 0.5 (Table S3). The Goodness-of-Fit (GoF) index yielded a moderate value of 0.3443. Among the endogenous variables, the plant quality construct showed moderate explanatory power ( $R^2 = 0.391$ ), while soil quality and soil fauna had weak explanatory power ( $R^2 = 0.16$  and  $R^2 = 0.154$ ). All analyses were conducted in R, using the “plspm” package<sup>59</sup>.

## Data availability

The data sets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

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## Author contributions

The concept of the manuscript was developed by I.G., M.C.-W., and M.Sz. The main manuscript was written by I.G., M.C.-W., S.Ś., M.Sz., T.Sz., and M.W.R. The methodology was developed by all authors. The experiments were conducted by I.G. (mesofaunal part), M.C.-W., S.Ś., M.Sz., T.Sz., and M.W.R. (vegetation part). The data analysis and visualization were performed by I.G., M.C.-W., S.Ś., T.Sz., and M.W.R. The review and editing were done by I.G. and M.C.-W.

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## Declarations

### Competing interests

The authors declare no competing interests.

### Ethics declaration

The appropriate permission was obtained for conducting experiments in protected areas.

### Additional information

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