# Recovery Signs in Grass-Stand Invertebrate Communities after a Decrease in Copper-Smelting Emissions

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**Abstract**—Invertebrate communities inhabiting the grass stand were studied during periods of relatively high (2006–2008) and almost nonexistent (2015–2017) emissions from Middle Ural Copper Smelter (MUCS) (main pollutants:  $SO_2$  and heavy metals; the total volume of emissions dropped between the periods by 75 times to 3000 t/yr). In 2015 and 2016, strong weather fluctuations were noted; potentially, such fluctuations could negate the recovery processes that had just begun. Still, in the moderately contaminated area, recovery signs were registered both in the grass stand (an increase in the phytomass of forbs by 1.5 times and its share in the total phytomass from 56 to 76%, as well as an increase in the similarity of the species structure with uncontaminated areas) and in invertebrate communities (the abundance of sucking herbivores decreased by 1.3 times; the abundance of cicadae, by 1.6 times; and the similarity between the background and buffer zones in trophic and taxonomic structures of communities increased). No recovery signs were detected in the severely contaminated area. The results confirm the hypotheses stating that in severely contaminated areas, meadow communities is stable; while in moderately contaminated areas, meadow communities recover relatively quickly.

**Keywords:** herbivores, predators, cicadae, bugs, beetles, spiders, meadow ecosystems, industrial pollution, heavy metals, emission reduction, natural recovery, resistance

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## **INTRODUCTION**

Invertebrates inhabiting the grass stand are traditionally considered a separate layer of the terrestrial fauna featuring high abundance and taxonomic richness; in addition, all its elements have close relationships with herbaceous vegetation [1]. Interest in this group is determined by the presence of representatives of most other terrestrial layers in its composition; with some caution, this makes it possible to extrapolate estimates of their resistance to stress impacts on the entire invertebrate population.

At present, atmospheric emissions decrease due to the modernization or closure of metallurgical enterprises [2]. In ecosystems surrounding such former pollution sources, this should result in the initiation and gradual development of recovery processes. Indeed, examples involving the restoration of plant communities [3–5], communities of herpetobiont invertebrates [6], and communities of grass stand dwellers [3] have been recorded. However, the studies cited describe a single and rather specific pollution source: a phosphate fertilizer plant whose operation resulted in alkalization of upper soil horizons (pH increased from 7 to 9). For lands in the vicinity of smelters contaminated by heavy metals, the continued digression of invertebrate communities is more typical; for instance, such digressions were described for the soil [7] and litter [8, 9] layers. Such data support the "inertial" hypothesis stating that biota remains in a depressed state for a long time even after the complete termination of emissions [10, 11]. Overall, the number of studies examining invertebrates in the context of decreasing emissions is very small so far [12].

Emissions from Middle Ural Copper Smelter have been gradually decreasing since the early 1990s and ceased almost completely after 2010 [13], which made it possible to examine ecosystem restoration patterns. In the most severely contaminated area, the Cu concentration decreases and pH normalizes in litter and upper soil horizons of forest sites [13], although no recovery signs are observed yet in the grass–dwarfshrub layer of forest ecosystems [14–16]. This makes it possible to suggest that recovery rates in meadow communities closely adjacent to these forest sites are low, too. Concurrently, recovery processes are likely to be more pronounced in the moderately contaminated area. Some prerequisites for this (e.g., an increase in the share of forbs), as well as possible recovery trends, were described in our earlier studies [17-19].

**Objective**—To analyze changes that occur in invertebrate communities of the meadow grass stand as a result of a decrease in copper-smelting emissions. The following hypothesis was tested: in moderately contaminated areas, a decrease in emissions results in a relatively rapid recovery of communities; while in severely contaminated areas, there is no recovery, or it is less pronounced.

#### MATERIALS AND METHODS

The studies were conducted in the vicinity of Middle Ural Copper Smelter (MUCS) located on the outskirts of the city of Revda, Sverdlovsk oblast. In 1980, coppersmelting emissions (SO<sub>2</sub> and heavy metals associated with dust particles) amounted to 225000 t/yr; in 1990, to 148000 t/yr; and in 2000, to 63000 t/yr. After the reconstruction in 2010, emissions have virtually ceased (some 3000 t/yr). The total mass of atmospheric emissions decreased by 75 times in the period from 1980 to 2012 (including a decrease in  $SO_2$  by 116 times (from 201000 to 1700 t/yr) and in dust particles by 44 times (from 21000 to 500 thousand t/yr)). Cu emissions dropped by 5500 times (from 4400 to 0.8 t/yr); As emissions, by 1571 times (from 900 to 0.6 t/yr); and Pb emissions, by 16 times (from 1000 to 70 t/yr). Zn emissions decreased by 15 times (from 1800 to 100 t/yr) in the period from 1989 to 2012. More detailed descriptions of the emission composition and dynamics are provided in earlier studies [13, 14].

The key sites are located in the western direction from the MUCS (against the prevailing wind direction) in the impact (1 km from the smelter, severe contamination), buffer (4 km, low contamination), and background (30 km, contamination corresponds to the regional background level) zones in topographically low relief elements on secondary dry meadows formed on forest glades some 5000 m<sup>2</sup> in size as a result of felling performed some 70 years ago. The rationale for this contamination-based zonation is addressed in detail in earlier studies [11, 13, 14]. The floristic composition of meadow vegetation varies significantly in different contamination zones due to the disappearance of sensitive forb species and their substitution with gramineous plants in the vicinity of the copper smelter: forb meadows in the background zone, forbgramineous meadows in the buffer zone, and gramineous meadows with the absolute predominance of Agrostis capillaris L. in the impact zone. A more detailed grass stand description was provided in an earlier work [17]. At the time of the study, all key sites were not used for grazing or haymaking.

Invertebrates inhabiting the grass stand were sampled using a modified Konakov-Onisimova biocoenometer (base area: 0.25 m<sup>2</sup>) combined with a portable suction sampler with an autonomous power source. Each sample is the result of a single biocoenometer installation with the subsequent collection of all invertebrates captured inside it and cutting off all herbaceous plants at the soil level. The first period of this study was 2006–2008; the second period, 2015– 2017. Each year, censuses were conducted in three rounds in the second half of each summer month (1st round in June, 2nd round in July, and 3rd round in August). Three sampling plots  $50 \times 50$  m in size were established in each contamination zone at a distance of 100–300 m from each other. The same permanent sampling plots were used in both periods of the study. The biocoenometer design [20] and sampling methodology and procedure [17] are described in detail in earlier studies.

The total size of the general sample was as follows: 10 samples per sampling plot per census round. Thus, 1620 samples of invertebrates and plants (270 per year) were collected over 6 years (18 rounds). More than 62600 invertebrate individuals were collected in the first period; more than 61 900, in the second period. For plants, the following parameters were measured accurate to 0.1 g: total air-dry weight and weights of the graminoid (cereals, sedges, and rushes) and forb fractions. Plant species were identified in materials collected in August 2008 and August 2015.

Taxonomic affiliation (to the family level) and trophic specialization of invertebrates were determined in the laboratory environment. In total, six trophic groups were examined: sucking and chewing herbivores, sucking and chewing predators, hemophages, and others (Table 1).

Data processing involved the computation of standard descriptive statistics (i.e., mean and standard error). Analysis of effects exercised by the contamination zone and the study period on the abundance of invertebrates and phytomass was performed using the LMERConvenienceFunctions package [21] based on generalized linear models with mixed effects (fixed factors: contamination zone and study period; random factor: sampling plot). Multiple comparisons were performed in the multcomp package [22] using the Tukey test.

The Standardized Precipitation–Evapotranspiration Index (SPEI) was computed in the SPEI package [23] for a set of mean monthly air temperatures and total monthly precipitation amounts for the period from January 1959 to December 2021 based on data collected at the Revda weather station (WMO ID 28430, [24]); the results were visualized in the ggplot2 package [25]. The SPEI reflects the ratio between pre-

| Trophic group        | Taxonomic or composite group  |  |  |  |  |  |
|----------------------|---|--|--|--|--|--|
| Sucking herbivores   | Heteroptera: Berytinidae, Coreidae, Lygaeidae, Miridae, Pentatomidae (except for Asopinae),<br>Rhopalidae, Scutelleridae, Tingidae  |  |  |  |  |  |
|                      | Auchenorrhyncha: Aphrophoridae, Cicadellidae, Cixiidae, Delphacidae, Membracidae  |  |  |  |  |  |
|                      | Sternorrhyncha: Psyllidae, Aphididae, Coccinea  |  |  |  |  |  |
|                      | Diptera: Brachycera anthophaga  |  |  |  |  |  |
|                      | Lepidoptera: Lepidoptera (i)  |  |  |  |  |  |
| Chewing herbivores   | Orthoptera: Acrididae   |  |  |  |  |  |
|                      | <b>Coleoptera</b> : Attelabidae, Apionidae, Brentidae, Buprestidae, Byrrhidae, Cerambycidae, Chrysomelidae, Curculionidae, Elateridae, Lagriidae, Mordellidae, Nitidulidae, Oedemeridae   |  |  |  |  |  |
|                      | Diptera: Nematocera anthophaga  |  |  |  |  |  |
|                      | Lepidoptera: Lepidoptera (l)  |  |  |  |  |  |
|                      | Hymenoptera: Symphyta   |  |  |  |  |  |
|                      | <b>Gastropoda</b> : Agriolimacidae, Arionidae, Bradybaenidae, Cochlicopidae, Discidae, Ellobiidae, Euconulidae, Gastrodontidae, Hygromiidae, Oxychilidae, Punctidae, Succineidae, Valloniidae, Vertiginidae, Vitrinidae   |  |  |  |  |  |
| Sucking predators    | Heteroptera: Anthocoridae, Nabiidae, Pentatomidae (Asopinae), Reduviidae, Saldidae  |  |  |  |  |  |
|                      | Neuroptera: Chrysopidae (l)   |  |  |  |  |  |
|                      | Diptera: Asilidae   |  |  |  |  |  |
|                      | Aranei: Araneidae, Clubionidae, Corinnidae, Dictynidae, Eutichuridae, Gnaphosidae,<br>Hahniidae, Linyphiidae, Liocranidae, Lycosidae, Mimetidae, Oxyopidae, Philodromidae,<br>Pisauridae, Salticidae, Sparassidae, Tetragnathidae, Theridiidae, Thomisidae, Zoridae |  |  |  |  |  |
| Chewing predators    | Odonata: Odonata (i)  |  |  |  |  |  |
|                      | Coleoptera: Cantharidae, Carabidae, Coccinellidae, Colydiidae, Lampyridae, Malachidae   |  |  |  |  |  |
|                      | Neuroptera: Chrysopidae (i)   |  |  |  |  |  |
|                      | Opiliones: Nemastomatidae, Phalangiidae   |  |  |  |  |  |
|                      | Lithobiomorpha: Lithobiidae   |  |  |  |  |  |
| Hemophages           | Diptera: Culicidae, Simuliidae, Tabanidae, Brachycera haemophaga  |  |  |  |  |  |
|                      | Ixodida: Ixodidae   |  |  |  |  |  |
| Other trophic groups | Blattoptera: Blattidae  |  |  |  |  |  |
|                      | Orthoptera: Tettigonidae  |  |  |  |  |  |
|                      | Heteroptera: Aradidae, Heteroptera indet. (1)   |  |  |  |  |  |
|                      | <b>Coleoptera</b> : Anthicidae, Catopidae, Helodidae, Hydrophilidae, Lathridiidae, Staphylinidae, Coleoptera (l), Coleoptera (p)  |  |  |  |  |  |
|                      | Hymenoptera: Apoidea, Vespoidea, Hymenoptera microparasitica  |  |  |  |  |  |
|                      | Diptera: Tipulidae, Diptera (l)   |  |  |  |  |  |
|                      | Insecta: Insecta indet. (l), Insecta indet. (p)   |  |  |  |  |  |
|                      | Nematoda  |  |  |  |  |  |
|                      | Annelida: Lumbricidae   |  |  |  |  |  |

Development stages: (i) imago, (l) larva, and (p) pupa.

Table 1. Composition of trophic groups

(b) (a) June July August 1.5 1.5 SPEI index 0 0 -1.5-1.5 2016 2006 2007 2008 2015 2017 2006 2007 2008 2015 2016 2017 Year of the study

Fig. 1. SPEI index in the studied years: (a) computed for each of the summer months; and (b) computed cumulatively for the four months (May–August). SPEI values higher than 1.5 indicate excessive moistening; values less than -1.5, a drought.

cipitation and potential evapotranspiration in any area on a global scale; its values can be substantially refined using local meteorological data collected over a fairly long period (30-50 years or more). The SPEI makes it possible to estimate moistening conditions during a given period (up to a month) in relation to the longterm average: values higher than 1.5 indicate excessive moistening; while values less than -1.5, a drought.

A dendrogram showing the species structure dissimilarity in plant communities was constructed using Ward's method in the pvclust package [26] based on the Bray-Curtis distance matrix. The structure of invertebrate communities (i.e., ratios between trophic and large taxonomic groups) was visualized in the vegan (metaMDS function, [27]) and ggord [28] packages using nonmetric multidimensional scaling (nMDS) based on Bray-Curtis distance matrices. The significance of differences in the community structure between zones and periods was estimated using permutational multivariate analysis of variance (PER-MANOVA; 999 permutations; adonis2 function in the vegan package) and post-hoc multiple comparisons in the pairwiseAdonis package [29]. All computations were performed in the R software environment [30].

## RESULTS

According to the SPEI dryness index, the summer of 2015 was one of the most over moistened in more than 60 years; by contrast, the summer of 2016, was one of the driest. Years of 2006–2008 and 2017 were close to the long-term average in terms of moisture availability (Fig. 1).

In the background zone, phytomass of herbaceous plants (both the total phytomass and phytomass of the two fractions) did not differ between periods (Tables 2 and 3). In the buffer zone, phytomass of graminoids decreased by 1.7 times, while the phytomass of forbs increased by 1.5 times between the periods. A similar trend was noted in the impact zone (1.5 and 4.0 times, respectively). In the first period, the total phytomass in the buffer zone did not differ from the background zone; in the second period, it increased 1.2 times due to forbs; while the phytomass of graminoids decreased to values close to the background ones. Differences in the species structure of grass stands reached the maximum between the periods; while differences between the contamination zones were less contrasting (Fig. 2).

The total abundance of invertebrates turned out to be similar in the first and second periods:  $309.7 \pm 14.9$ and  $306.0 \pm 21.7$  ind./m<sup>2</sup>, respectively. In the background zone, the total abundance of invertebrates, as well as the abundance of predators, did not differ between periods (Tables 2, 3). In the buffer zone, the abundance of chewing herbivores and predators did not differ between periods; while in the impact zone, the abundance of all trophic groups was different in the first and second study periods.

A comparison of the background and buffer zones indicates that the total abundance differed in the first period and did not differ in the second period (Tables 2 and 3). The disappearance of differences is due to a decrease in the abundance of sucking herbivores in the buffer zone by 1.3 times, including the two most abundant groups: cicadae (1.6 times) and bugs (1.3 times). This trend was also noted for predatorous bugs (their abundance decreased by 1.7 times). Overall, the number of trophic and taxonomic groups featuring no differences between the background and buffer zones significantly increased in the second period. Phytophagous beetles were an exception: their abundance decreased in the second period in both zones, and in the background zone it dropped much more (by 3.7 times) than in the buffer zone (by 1.8 times), which makes these differences statistically

|                                     | Study period and contamination zone |                                    |                                    |                                   |                                    |                                    |  |  |  |  |  |
|-------------------------------------|-------------------------------------|------------------------------------|------------------------------------|-----------------------------------|------------------------------------|------------------------------------|--|--|--|--|--|
| Trophic group/Phytomass<br>fraction | Ι                                   |                                    |                                    |                                   | II                                 |                                    |  |  |  |  |  |
| nuction                             | background                          | buffer                             | impact                             | background                        | buffer                             | impact                             |  |  |  |  |  |
| Invertebrates                       |                                     |                                    |                                    |                                   |                                    |                                    |  |  |  |  |  |
| Total abundance                     | $227.9 \pm 11.2$                    | $\underline{297.8 \pm 13.6}$       | $\underline{403.6\pm33.3}$         | $\underline{223.7 \pm 18.0}$      | $221.8 \pm 16.2$                   | $\underline{472.6 \pm 45.9}$       |  |  |  |  |  |
| Total abundance                     | 213–237                             | 243–333                            | 275-485                            | 164–285                           | 184–277                            | 420–532                            |  |  |  |  |  |
| Sucking herbivores                  | $\frac{95.9 \pm 7.5}{92 - 103}$     | $\frac{161.0 \pm 10.6}{139 - 172}$ | $\frac{328.3 \pm 31.2}{195 - 400}$ | $\frac{132.6 \pm 15.8}{76 - 204}$ | $\frac{126.2 \pm 14.0}{92 - 185}$  | $\frac{415.1 \pm 46.3}{363 - 503}$ |  |  |  |  |  |
| cicadae                             | $\frac{63.9 \pm 6.1}{56 - 72}$      | $\frac{116.6 \pm 8.6}{91 - 140}$   | $\frac{283.1 \pm 30.1}{152 - 351}$ | $\frac{89.2 \pm 12.8}{36 - 142}$  | $\frac{73.3 \pm 10.5}{44 - 108}$   | $\frac{377.5 \pm 45.2}{318 - 467}$ |  |  |  |  |  |
| herbiyorous bugs                    | $11.9 \pm 1.8$                      | $20.4 \pm 4.0$                     | 132-331<br>$25.0 \pm 4.3$          | $13.8 \pm 2.0$                    | $14.8 \pm 1.7$                     | $20.3 \pm 4.7$                     |  |  |  |  |  |
|                                     | 9–16                                | 14-30                              | 20-28                              | 10-20                             | 11-20                              | 6-31                               |  |  |  |  |  |
| herbivorous Brachycera              | $\frac{13.2 \pm 1.4}{12 - 14}$      | $\frac{16.9 \pm 2.1}{13-23}$       | $\frac{12.1 \pm 1.3}{10-16}$       | $\frac{25.0 \pm 3.5}{14-34}$      | $\frac{33.8 \pm 5.4}{17 - 51}$     | $\frac{13.0 \pm 1.3}{8-19}$        |  |  |  |  |  |
| Chewing herbivores                  | $\frac{45.2 \pm 3.0}{41 - 52}$      | $\frac{46.1 \pm 2.9}{38 - 54}$     | $\frac{11.0 \pm 1.3}{7 - 14}$      | $\frac{33.9 \pm 2.8}{26 - 46}$    | $\frac{40.1 \pm 3.8}{29 - 59}$     | $\frac{16.7 \pm 4.2}{4-39}$        |  |  |  |  |  |
| herbivorous beetles                 | $\frac{5.5 \pm 0.5}{5-6}$           | $\frac{8.6 \pm 1.2}{5 - 12}$       | —                                  | $\frac{1.5 \pm 0.4}{1-2}$         | $\frac{4.9 \pm 1.1}{2 - 10}$       | —                                  |  |  |  |  |  |
| herbivorous Nematocera              | $\frac{10.2 \pm 1.8}{7 - 16}$       | $\frac{9.2 \pm 1.2}{6 - 12}$       | $\frac{4.4 \pm 1.1}{1-6}$          | $\frac{18.1 \pm 2.2}{12 - 28}$    | $\frac{24.9 \pm 3.8}{12 - 42}$     | $\frac{14.7 \pm 3.9}{3-34}$        |  |  |  |  |  |
| mollusks                            | $\frac{16.4 \pm 1.9}{10 - 21}$      | $\frac{13.0 \pm 1.5}{7 - 19}$      | _                                  | $\frac{6.8 \pm 1.0}{5-10}$        | $\frac{3.6 \pm 0.9}{1-7}$          | _                                  |  |  |  |  |  |
| Sucking predators                   | $\frac{25.8 \pm 1.4}{24 - 28}$      | $\frac{32.6 \pm 2.3}{29 - 38}$     | $\frac{16.3 \pm 1.5}{12 - 22}$     | $\frac{24.1 \pm 2.4}{16 - 34}$    | $\frac{20.4 \pm 1.3}{16 - 24}$     | $\frac{7.7 \pm 0.6}{6-9}$          |  |  |  |  |  |
| predatorous bugs                    | $\frac{3.9 \pm 0.5}{3-4}$           | $\frac{6.8 \pm 0.8}{6-8}$          | $\frac{2.7 \pm 0.5}{0-4}$          | $\frac{5.6 \pm 0.8}{3-8}$         | $\frac{3.9 \pm 0.6}{1-6}$          | $\frac{0.4 \pm 0.2}{0-1}$          |  |  |  |  |  |
| spiders                             | $\frac{22.2 \pm 1.2}{21 - 24}$      | $\frac{26.4 \pm 2.0}{23 - 32}$     | $\frac{14.1 \pm 1.3}{11 - 20}$     | $\frac{19.0 \pm 2.0}{14 - 26}$    | $\frac{15.4 \pm 1.3}{12 - 18}$     | $\frac{6.8 \pm 0.5}{5-8}$          |  |  |  |  |  |
| Chewing predators                   | $\frac{5.8 \pm 0.8}{5-6}$           | $\frac{3.4 \pm 0.7}{3-4}$          | $\frac{0.1 \pm 0.1}{0-1}$          | $\frac{4.4 \pm 0.7}{4-5}$         | $\frac{3.0 \pm 0.6}{1-5}$          | _                                  |  |  |  |  |  |
| predatorous beetles                 | $\frac{1.3 \pm 0.4}{1-2}$           | $\frac{1.2 \pm 0.5}{1-1}$          | $\frac{0.1 \pm 0.1}{0-1}$          | $\frac{0.3 \pm 0.2}{0-1}$         | $\frac{0.6 \pm 0.3}{0-1}$          | _                                  |  |  |  |  |  |
| harvestmen                          | $\frac{3.9\pm0.8}{4-4}$             | $\frac{1.2 \pm 0.4}{1-2}$          | _                                  | $\frac{3.6 \pm 0.7}{3-4}$         | $\frac{1.5 \pm 0.6}{0-2}$          | _                                  |  |  |  |  |  |
| Hemophages                          | $\frac{36.9 \pm 10.9}{14 - 57}$     | $\frac{31.3 \pm 6.6}{20 - 38}$     | $\frac{20.7 \pm 6.8}{15 - 30}$     | $\frac{11.9 \pm 4.0}{3-28}$       | $\frac{10.4 \pm 2.6}{3-16}$        | $\frac{6.7 \pm 1.5}{1-10}$         |  |  |  |  |  |
| Other groups                        | $\frac{18.8 \pm 1.6}{12 - 22}$      | $\frac{23.7 \pm 2.2}{15 - 30}$     | $\frac{27.0 \pm 2.3}{21 - 35}$     | $\frac{17.5 \pm 1.5}{14 - 22}$    | $\frac{23.1 \pm 2.2}{17 - 31}$     | $\frac{26.7 \pm 3.1}{17 - 32}$     |  |  |  |  |  |
| Herbaceous plants                   |                                     |                                    |                                    |                                   |                                    |                                    |  |  |  |  |  |
| Total phytomass                     | $\frac{219.4 \pm 10.1}{200 - 236}$  | $\frac{236.4 \pm 7.8}{216 - 249}$  | $\frac{149.9 \pm 5.5}{128 - 171}$  | $\frac{209.9 \pm 9.0}{194 - 220}$ | $\frac{252.3 \pm 13.4}{240 - 266}$ | $\frac{104.9 \pm 4.8}{86 - 115}$   |  |  |  |  |  |
| Graminoids                          | $\frac{63.6 \pm 7.2}{44 - 86}$      | $\frac{104.3 \pm 7.4}{84 - 125}$   | $\frac{149.0\pm5.6}{126170}$       | $\frac{60.1 \pm 3.5}{52 - 72}$    | $\frac{60.6 \pm 6.2}{41 - 96}$     | $\frac{101.2 \pm 4.3}{84 - 113}$   |  |  |  |  |  |
| Forbs                               | $\frac{156.6 \pm 7.9}{137 - 194}$   | $\frac{132.7 \pm 7.0}{112 166}$    | $\frac{0.9\pm0.4}{0-3}$            | $\frac{150.5 \pm 8.0}{143 165}$   | $\frac{192.0 \pm 13.0}{171 - 210}$ | $\frac{3.6 \pm 1.4}{0-9}$          |  |  |  |  |  |

Table 2. Abundance of invertebrates (ind./ $m^2$ ) and plant phytomass (g/ $m^2$ ) in grass stands of the studied meadows

Note: the accounting unit is sampling plot; above the line is the mean value  $\pm$  standard error, n = 27; below the line is the range of mean annual abundance values for the study period, n = 9; a dash indicates that the group was not found in the impact zone.



Contamination zone and year of the study

**Fig. 2.** Species structure dissimilarity in grass stands in the background (B), buffer (Bf), and impact (I) contamination zones in different years of the study. Figures at the bases of dendrogram branches are approximately unbiased support numbers (AU, %). Clusters with AU  $\ge$  95% are considered statistically significant.

**Table 3.** Results of multiple comparisons performed for generalized linear models with mixed effects (fixed factors: contamination zone and study period; random factor: sampling plot) describing the abundance of invertebrate trophic groups inhabiting the grass stand and phytomass of various fractions of meadow herbaceous vegetation

|                                  | Study period and contamination zone |         |         |                           |         |         |         |         |         |
|----------------------------------|-------------------------------------|---------|---------|---------------------------|---------|---------|---------|---------|---------|
| Group                            | Differences inside zones            |         |         | Differences between zones |         |         |         |         |         |
|                                  | B1-B2                               | Bf1-Bf2 | I1–I2   | B1-Bf1                    | B2–Bf2  | Bf1–I1  | Bf2–I2  | B1-I1   | B2-I2   |
| Total abundance of invertebrates | 0.995                               | < 0.001 | < 0.001 | < 0.001                   | 1.000   | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Sucking herbivores               | < 0.001                             | < 0.001 | < 0.001 | < 0.001                   | 0.996   | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Cicadae                          | < 0.001                             | < 0.001 | < 0.001 | < 0.001                   | 0.512   | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Herbivorous bugs                 | 0.921                               | 0.137   | 0.446   | 0.002                     | 0.997   | 0.540   | 0.189   | < 0.001 | 0.060   |
| Herbivorous Brachycera           | < 0.001                             | < 0.001 | 0.997   | 0.636                     | 0.176   | 0.340   | < 0.001 | 0.997   | < 0.001 |
| Chewing herbivores               | 0.012                               | 0.545   | 0.049   | 1.000                     | 0.660   | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Herbivorous beetles              | 0.002                               | 0.070   | _       | 0.369                     | 0.014   | _       | _       | _       | _       |
| Herbivorous Nematocera           | 0.002                               | < 0.001 | < 0.001 | 0.994                     | 0.251   | 0.026   | 0.007   | 0.005   | 0.764   |
| Mollusks                         | < 0.001                             | < 0.001 | —       | 0.610                     | 0.092   | —       | —       | —       | —       |
| Sucking predators                | 0.991                               | < 0.001 | < 0.001 | 0.183                     | 0.696   | < 0.001 | < 0.001 | 0.002   | < 0.001 |
| Predatorous bugs                 | < 0.001                             | < 0.001 | < 0.001 | < 0.001                   | 0.127   | < 0.001 | < 0.001 | 0.415   | < 0.001 |
| Spiders                          | < 0.001                             | < 0.001 | < 0.001 | 0.245                     | 0.212   | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Chewing predators                | 0.855                               | 0.996   | _       | 0.339                     | 0.711   | 0.018   | —       | 0.003   | _       |
| Predatorous beetles              | 0.344                               | 0.842   | _       | 1.000                     | 0.975   | 0.418   | _       | 0.338   | _       |
| Harvestmen                       | 0.992                               | 0.964   | _       | 0.061                     | 0.210   | _       | _       | —       | _       |
| Hemophages                       | < 0.001                             | < 0.001 | < 0.001 | 0.978                     | 0.997   | 0.287   | 0.460   | 0.058   | 0.213   |
| Other trophic groups             | 0.993                               | 1.000   | 1.000   | 0.374                     | 0.198   | 0.842   | 0.780   | 0.023   | 0.005   |
| <b>Total phytomass</b>           | 0.840                               | 0.421   | < 0.001 | 0.298                     | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Graminoid phytomass              | 0.963                               | 0.001   | < 0.001 | < 0.001                   | 1.000   | 0.001   | < 0.001 | < 0.001 | < 0.001 |
| Forb phytomass                   | 0.929                               | < 0.001 | 0.020   | 0.139                     | 0.005   | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

Significance levels (p) are provided for a sample collected on 3 sampling plots (n = 3). Contamination zones: (B) background, (Bf) buffer, and (I) impact. A dash indicates that the group was not found in the impact zone.



**Fig. 3.** Ordination of the abundance of main trophic groups of invertebrates inhabiting the grass stand: (a) in the first period of the study; and (b) in the second period of the study. Groups: (Abun) total abundance, (Su\_Hbv) sucking herbivores, (Chw\_Hbv) chewing herbivores, (Su\_Prd) sucking predators, and (Chw\_Prd) chewing predators. Contamination zones: (B) background, (Bf) buffer, and (I) impact.

significant. A comparison of the background and buffer zones with the impact zone revealed statistically significant differences in the total abundance both in the first and second periods due to the persistently high abundance of sucking herbivores (including cicadae) in the most severely contaminated area. A similar trend was noted for all trophic and most taxonomic groups.

In different contamination zones, the structure of invertebrate communities manifested in the ratio between trophic and taxonomic groups was different in the first and second study periods. In the first period, the ratio between trophic groups (Fig. 3) (number of ordination diagram measurements: 2; stress: 0.061) differed between all contamination zones (p = 0.001). In the second period (number of measurements: 2; stress: 0.098), there were no differences between the background and buffer zones (p = 0.804); however, the differences remained when comparing the impact zone with other zones (p = 0.001). The average distance to centroids increased from the first to the second period for all zones: in the background zone, from 0.120 to 0.192; in the buffer zone, from 0.108 to 0.175; and in the impact zone, from 0.194 to 0.241.



**Fig. 4.** Ordination of the abundance of large taxonomic groups of invertebrates inhabiting the grass stand: (a) in the first period of the study; and (b) in the second period of the study. Groups: (Hrb\_beetles) herbivorous beetles, (Hrb\_bugs) herbivorous bugs, (Hrb\_brach) herbivorous Brachycera, (Hrb\_hopp) cicadae, (Hrb\_moll) mollusks, (Hrb\_nemat) herbivorous Nematocera, (Prd\_beetles) predatorous beetles, (Prd\_bugs) predatorous bugs, (Prd\_Harv) harvestmen, and (Prd\_spiders) spiders. See Fig. 3 for impact zone symbols and colors.

The ratio between taxonomic groups (Fig. 4) changed in a similar way: in the first period (number of measurements: 2, stress: 0.120), all zones differed from each other (p = 0.001). In the second period (number of measurements: 2, stress: 0.160), the differences between the background and buffer zones disappeared (p = 0.192), but remained when comparing the impact zone with other zones (p = 0.001). The average distance to centroids also increased for all zones from

the first to the second period: in the background zone, from 0.220 to 0.280; in the buffer zone, from 0.188 to 0.273; and in the impact zone, from 0.234 to 0.287.

In both periods, the total abundance of invertebrates, as well as the abundance of sucking herbivores, cicadae, and herbivorous bugs, correlated positively with each other and with the contamination level. The abundance of chewing herbivores and herbivorous beetles, and mollusks correlated negatively with the contamination level; a similar trend was demonstrated by sucking predators (predatorous bugs) in the second period. Chewing predators (similar to sucking predators) showed a weak relationship with the contamination level.

## DISCUSSION

An important result of this study is the absence of differences in the total abundance of invertebrates between the first and second periods in the background zone (Tables 2 and 3). Pronounced weather differences between the study periods were noted earlier [19]. The extension of the interval of years used to compute the SPEI dryness index by 1/3rd confirmed this fact (Fig. 1). Despite the pronounced weather fluctuations, the invertebrate population inhabiting the grass stand remained generally stable. Concurrently, the distance to centroids in the ordination diagrams increased in the second period, which reflects the presence of a destabilizing effect and is consistent with the above-described weather anomalies in the second period.

The stability of the invertebrate population in the background zone suggests that the differences between periods noted in contaminated areas are primarily determined by the decrease in pollution that has initiated recovery processes in the grass stand, including an increase in plant diversity, a decrease in the share of graminoids, and softening of the microclimate that used to be shifted towards a greater aridity degree [18, 19].

A comparison of our data with data collected during the period of intensive MUCS emissions (1988–1989 [11]) shows a significant increase in the phytomass of forbs in the buffer zone: compared to 1988–1989, it increased by 1.5 times in the first period and by 2.2 times in the second period; the total phytomass increased in these periods as well (by 1.1 and 1.4 times, respectively). The change in the ratio between fractions in the total phytomass is an indicative sign: the proportion of forbs increased from 50%in 1989 to 56% in the first period and to 76% in the second period. As a result, the phytomass structure in the buffer zone in the second period became similar to that in the background zone. The grass stand species structure there also became more similar to the background zone (Fig. 2). These changes can be considered a manifestation of recovery processes in the herbaceous vegetation layer.

By contrast, phytomass of graminoids in the impact zone has significantly decreased in comparison with 1988–1989; as a result, the total phytomass also dropped by 2 and 3 times in the first and second periods. Despite a fourfold increase in the forb phytomass in the second period compared to the first period (Table 2), the absolute phytomass values remained

negligible; as a result, the ratio between proportions of various fractions in the total phytomass did not change in the impact zone.

In the above-mentioned work [11], the approach used to distinguish trophic groups of invertebrates was somewhat different from the one used in this study. which complicates the direct data comparison. Still, for several parameters, it turned out to be possible.

For instance, our data indicate that the total abundance of invertebrates in the grass stand in the buffer zone increased in the first period by 1.6 times compared to 1989; however, in the second period, it almost returned to its original values due to a decrease in the abundance of several groups (primarily sucking herbivores: cicadae and bugs). Importantly, the abundance values (total abundance, abundance of sucking herbivores, and abundance of cicadae) have approached in the second period those registered in the background zone (Tables 2 and 3, Fig. 3). Sucking herbivores are one of the few groups whose abundance often increases in contaminated areas [31]. Our data confirm the pronounced positive relationship between this group and the contamination degree (Figs. 3 and 4). Therefore, a decrease in the abundance of sucking herbivores can be considered, with some caution, a sign of recovery dynamics after a decrease in emissions. In the buffer zone, shares of sucking herbivores and cicadae in the total abundance decreased from 86% and 80% in 1989 to 56% and 39% in the first period and to 53% and 33% in the second period, respectively, which also indicates the recovery of communities. It must be noted that a decrease in shares of these groups was registered as early as in the first period (i.e., prior to an almost complete termination of emissions in 2010).

In the impact zone, the total abundance of invertebrates was steadily increasing: by 1.4 times in the first period compared to 1989 and by 1.7 times in the second period. This increase is determined by the growing proportion of cicadae: from 60% in 1989 to 70% in the first period and up to 80% in the second period. The share of sucking herbivores in these periods amounted to 88, 81, and 88%, respectively. The steadily high abundance of sucking herbivores (in particular, cicadae) in the most severely contaminated area in both study periods also indirectly indicates the presence of recovery processes in the buffer zone.

Overall, the performed analysis of the invertebrate population inhabiting the meadow grass stand (including the total abundance of invertebrates and the abundance of and ratio between their trophic and taxonomic groups) indicates a clearly manifested recovery trend in the moderately contaminated area and no recovery dynamics on severely contaminated sites. This conclusion is consistent with the inertial hypothesis [10, 14, 32] stating that ecosystems in the vicinity of the enterprise remain in the depressed state for a long time even after the complete termination of emissions, and natural recovery processes begin in them with a delay and are initially weakly manifested.

In addition to MUCS, the recovery of meadow communities was examined only in the vicinity of a phosphate fertilizer plant in Germany. A rapid (within 10 years after the termination of emissions) and pronounced increase in the diversity of meadow plants [3–5] and in species richness of herpetobionts [6] and invertebrates inhabiting the grass stand [3] was demonstrated. However, in that case, the plant's emissions had alkalized the environment, which is radically different from the MUCS impact that contaminates ecosystems with heavy metals and concurrently acidifies the environment. For such an emission structure, literature sources describe either the complete absence of recovery signs or only initial recovery stages.

Examples showing the complete absence of recovery dynamics in invertebrate communities are known for soil macrofauna (15 years after a decrease in emissions) [7] and for herpetobiont communities (30 years after a decrease in emissions) [8, 9]. In situations when recovery signs are registered, this process takes decades, which is also in good agreement with the inertial hypothesis. Ten-year-long monitoring carried out during the MUCS emission reduction stage revealed signs indicating the restoration of trophic activity of birch phyllophages: this was explained by a decrease in concentrations of heavy metal in leaves [33]. Individual recovery signs (e.g., appearance of new species) were noted for the mollusk population in the meadow grass stand in the moderately contaminated area in the vicinity of the MUCS [19]. For soil macrofauna, an increase in the total abundance of earthworms, enchytraeids, and mollusks and their advancement closer to the smelter was noted at the last stage of a 25-year-long monitoring study conducted on severely contaminated sites in the same area [34]. The recolonization of the area by these groups can be explained, among other things, by their spread from survival microsites (i.e., large dead trunks at late decomposition stages) [35]. The advancement of earthworms caused a shift in the European mole distribution limit towards the smelter 15–18 years after the decrease in emissions [36].

## CONCLUSIONS

Parameters of the meadow grass stand and invertebrate communities inhabiting it during periods of relatively high (2006–2008) and almost nonexistent (2015–2017) emissions from a major copper smelter were estimated. Strong weather fluctuations were noted in the second period; potentially, such fluctuations could adversely affect the studied communities and negate the recovery processes that had just begun in them. Still, in the moderately contaminated area. recovery signs were registered both in the grass stand (an increase in forb phytomass, changes in the ratio between phytomass fractions, and changes in the species structure) and in invertebrate communities (the abundance of sucking herbivores, including cicadae, decreased; while the similarity between the trophic and taxonomic structures of communities in moderately contaminated and background areas increased). Taking that the abundance and structure of invertebrate communities in the background zone remained virtually unchanged between the study periods, the above-described changes can be interpreted as recovery. No recovery signs were detected in the severely contaminated area. The described situation is consistent with the inertial hypothesis and the initial assumption about the relatively rapid recovery of meadow communities affected by moderate contamination.

Such results were obtained for invertebrate communities inhabiting the grass stand for the first time. Further studies of the species structure of grass-stand invertebrate communities after a decrease in emissions are of utmost interest; such studies will make it possible, among other things, to separate the recovery trend from weather fluctuations in a more reliable way. In addition, in the context of the current widespread reduction in industrial emissions, it seems to be promising to expand the geographic reach of such studies and analyze distinct community recovery features in various natural zones.

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## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of Interest

The author declares that he has no conflict of interest.

#### Statement on the Welfare of Animals

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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