The Use of Oribatid Mites and Diatoms as Combined Indicators of Contaminations from Multiple Origins in Riparian Zone Forest Soils in Estonia

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Abstract

This study investigates the combined use of oribatid mites and diatoms in pine forest riparian zone soils. We focus on these organism groups as bioindicators of long-term anthropogenic disturbances of various origins categorized as two contamination levels: 1) moderate level that integrates the effects of sulphate-rich mining water contamination and alkaline air pollution that affected the area in the 1980s; 2) mild level that has been affected only by the alkaline air pollution. Additionally, the oribatid mite and diatom communities of these two groups of study sites were compared to another study area, which has similar natural conditions but is uninfluenced by these anthropogenic pollutants.

Changes in oribatid mite abundance and the presence or absence of specific diatom species were the most significant differences in comparison of the two contamination levels and the area with very low human impact. Based on the community dissimilarities, both bioindicators had difficulty differentiating between the contamination levels when viewed separately. However, when the community data of the two groups were pooled, their ability to indicate the studied contamination levels improved noticeably. The sulphate-rich mining water contamination showed no strong influence on the communities, therefore the full indicative potential of these two groups may not have been reached. Nevertheless, this study provided new information about the soil communities inhabiting riparian areas and through that helped us understand some of the dynamics in diatom and oribatid mite communities brought on by long-term but weak disturbances. Some promising species were proposed from both groups that might help predict the effects of sulphate-rich mining water contamination or alkaline air pollution. Also, the taxonomic list of oribatid mites provides a valuable insight into the Estonian riparian forest soil oribatid fauna.

Keywords: oribatid mites, diatoms, riparian zone, disturbances, anthropogenic influence.

Introduction

Oribatid mites (Acari: Oribatida) and diatoms (Bacillariophyceae) are microscopic, highly species rich and diverse components of the soil biota and are known to respond to environmental changes in their habitat. Oribatid mites, mostly known for being the dominant microarthropod group in the organic horizons of many soil ecosystems (Norton 1990), have previously been studied in relation to, e.g. air quality (e.g. Kehl and Weigmann 1992, Seniczak et al. 2002), trace metal contamination (e.g. Ivan and Vasiliu 2009, Skubała and Kafel 2004), and forest management (e.g. Farská et al. 2014, Lindo and Visser 2004). Also, the bioindication abilities of semi-aquatic oribatid mites have been studied, addressing various disturbances such as trampling (e.g. Borcard and Matthey 1995) and draining (e.g. Markkula 1986). However, a wide range of topics has still not been addressed, leaving vast knowledge gaps in their habitat preferences, ecology and sensitivity or tolerance towards various disturbances (Lebrun and Van Straalen 1995). Diatoms and their bioindication abilities have been almost exclusively studied in marine and freshwater ecosystems (e.g. Desrosiers et al. 2013, Hall and Smol 2010) but only on rare occasions from soil (e.g. Heger et al. 2012, Kabirov and Gaisina 2009, Vacht et al. 2014, van Kerckvoorde et al. 2000) and wetland habitats (e.g. Hargan et al. 2014, Poulíčková 2013).

Even though oribatid mites have been investigated more than diatoms living in the soil, the information about

both groups as bioindicators of anthropogenic disturbances in riparian habitats is scarce. In paleolimnological studies, two groups have been previously used together as bioindicators (e.g Luoto et al. 2009), though in these multi-proxy studies the full indicative potential of oribatid mites is often overlooked due to their low abundance in lake sediments (Solhøy and Solhøy 2000).

Riparian areas, which are known as biodiversity 'hotspots', are affected by changes occurring in catchment areas as well as in bodies of water through water-level fluctuations and water seepage (Grobbelaar 1983, Weilhoefer and Pan 2007). More specifically, riparian areas provide an ideal study system for observing disturbances from various origins, e.g. water contamination and air pollution. Therefore, the advantages of both bioindicator groups, diatoms, well-known aquatic indicators, and oribatid mites, more known for terrestrial bioindication abilities, can be combined here. Understanding the factors controlling the functioning of the riparian soil biota is also considered fundamental for restoration purposes (Fonseca and Joner 2007).

The present study investigates the effects of sulphate-rich mining waters pumped into a number of lakes in NE Estonia (Kurtna study area) and compares these results with other riparian zones in the vicinity, unaffected by the mining waters. These anthropogenic disturbances differ from many commonly studied human influences because even though the contaminants have been present for a long time (>50 years) (Rooma 1987) their levels can be considered mild. In addition, results from the contaminated Kurtna study area are compared with an area with a very low human influence located in SE Estonia (Mustoja study area).

Based on the knowledge about both bioindicator groups in question and numerous studies pointing out the benefits of multi-proxy approaches in indicating contamination effects and other human influences on ecosystems (Birks and Birks 2006, Lamentowicz 2015), the main hypothesis was proposed: Integrated use of diatom and oribatid mite community patterns provides more accurate bioindication than the two groups separately.

In order to investigate the integrated use of diatoms and oribatid mites as combined bioindicators of sulphate-rich mining water contamination and alkaline air pollution, the following goals were set: (1) to analyse the oribatid mite community structure and properties (e.g. species richness, diversity and evenness) and the variation of these properties depending on the level of anthropogenic influence; (2) to compare these results to diatom community data that has been previously published (Vacht et al. 2014); (3) to assess the benefits of using diatoms and oribatid mites as combined bioindicators.

Materials and Methods

Site description

The study areas are located in two kame fields: Kurtna, located in NE Estonia (59°16' N, 79 27°34' E), representing the contaminated area (Figure 1); and the area with a very low human influence, Mustoja, in SE Estonia (57°53' N 27°39' E). Kurtna kame field was affected by alkaline air pollution with elevated levels of trace metals in the 1980s; the effects of this can be seen in the moderately elevated soil pH values (Kont et al. 1994, 2007; Vacht et al. 2014). The sulphate content in a number of lakes (sites N1, N2, A, S and PK) in the Kurtna study area has increased due to pumping of mining waters into the area (Terasmaa et al. 2013). Based on this the study sites (Figure 1) can be divided into two groups based on their contamination levels: (1) two of the Kurtna study sites (J and M) that have been affected by alkaline air pollution, where the sulphate levels do not exceed 20 mg L^{-1} (Terasmaa et al. 2013) and can, therefore, be considered as mildly contaminated sites; (2) five of the Kurtna study sites (PK, S, A, N2 and N1) that have been affected by alkaline air pollution and the mining water contamination, where sulphate levels range from 160 to 270 mg L^{-1} (Terasmaa et al. 2013) and can be considered as moderately contaminated sites. In addition, three sites in Mustoja kame field (M1, M2 and M3) which is an area with no alkaline air pollution and no elevated sulphate content. Omitting the possibility that the community differences between two study areas could be induced by additional factors (e.g. water body type), the Mustoja study area was only used for comparison and not included in gradient analysis. All of the study sites at Kurtna and Mustoja had a similar hydric topsoil, ranging from gleyic podzols to histosols. The results of the analysis of the soil parameters measured (Vacht et al. 2014) are listed in Table 1.

The vegetation on both kame fields belongs to the oligo-mesotrophic boreal forest type (Paal 1997) and *Vaccinium myrtillus* boreal forest site type alternating with patches of drained wetlands characterized by *Ledum palustre* L. and *Pinus sylvestris* L. The vegetation at the sampling sites reflected the variable moisture conditions of the riparian soils, containing *Alnus incana* L., *Equisetum fluviatile* L. and *Sphagnum* mosses. Average yearly precipitation varies between 600 and 700 mm, exceeding the evaporation rate, and average yearly temperature ranges from 4.7 to 5.7 °C on both study areas (Estonian Environmental Agency 2014).

Sampling and chemical analyses

Soil microarthropods were sampled using a soil corer (196 cm^3, 10 cm depth) in September of 2012 and 2013. Microarthropod sampling was done in five repetitions

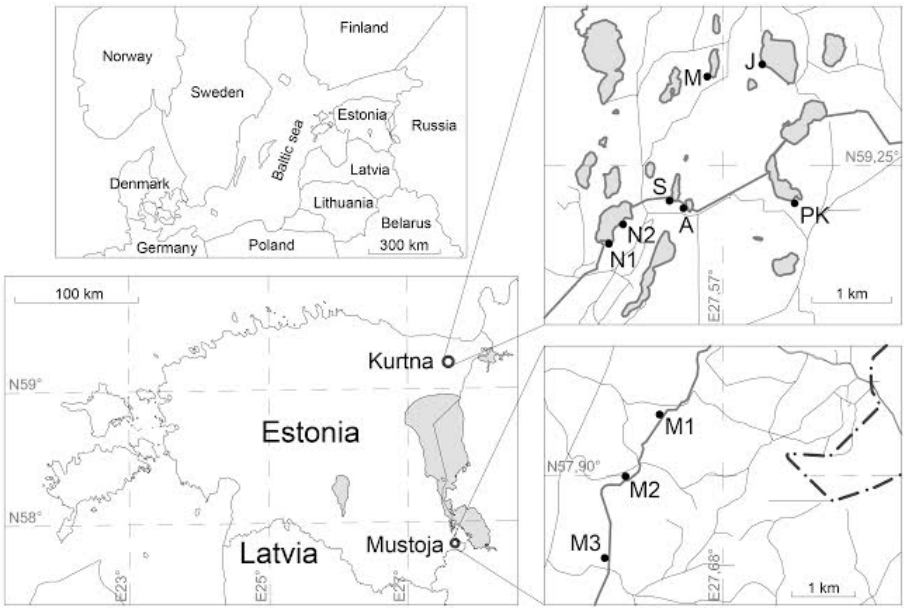

Figure 1. Locations of the study areas and study sites

from each site, parallel to the lake or stream shore, approximately 1–2 m from the water, where the effects of water logging were clearly visible through soil moisture and vegetation characteristics. A total of 100 microarthropod samples were collected. Oribatid mites were extracted by modified Berlese-Tullgren funnels until samples were dry (at least 48 hours). Adult oribatid mites were counted and identified to the species level, where damages made this impossible, to the genus level (Weigmann 2006). Juvenile oribatid mites were excluded from the analysis. The preparation of 55 diatom samples collected in 2012 has been previously described in detail (Vacht et al. 2014).

Samples for the analysis of soil properties were collected in September 2013. Standard methods were used to determine soil organic matter and carbonate content (Heiri et al. 2001) and soil pH_{H2O}. Total nitrogen, total carbon and exchangeable phosphorus contents were measured using AL extraction; potassium, calcium and magnesium by AL extraction using the MP-AES. These analyses were conducted from a pooled sample from each Kurtna study site and one pooled sample combining the sites in Mustoja.

Data analyses

Microarthropod data from both 2012 and 2013 was pooled for statistical analysis. Communities were compared by species richness, diversity (Shannon's *H'*) and evenness (Shannon's *J'*). Due to high variability in the abundance of oribatid mites between sampling sites, rarefied species richness was used to add a comparability measure to the observed species richness (Heck et al. 1975, Oksanen 2013a).

The effect of various disturbance levels (very low human influence, mildly contaminated and moderately contaminated) on community parameters and species abundance was tested using ANOVA. Levene's test for homogeneity of variances was used prior to ANOVA. The differences between the parameters and species abundance of the mildly and moderately disturbed communities were determined by the Kruskal-Wallis test. The same test was used to compare the community parameters and species abundances of the two study areas. Spearman correlation (ρ) was used to assess possible associations between community parameters, abundances of specific species and environmental parameters. These exploratory statistical analyses were done including all the identified species and measured environmental parameters. The relationship between the studied communities and the environmental parameters in Kurtna was investigated in more detail to understand the effects of sulphate rich mining water on riparian soil communities. For this purpose, the covariability and significance of each variable was considered and a selection was made amongst them: oribatid mite species composing more than 0.05% of the total abundance (18 species) and the potentially indicative diatom species (19 species) (Vacht et al. 2014) were chosen. These data, using the Hellinger transformation, along with six environmental parameters (organic matter, total nitrogen, exchangeable phosphorous, carbonate and potassium content in soil and sulphate content in the lake water) were included in the Canonical Correspondence Analysis (CCA) (Ter Braak 1986). This enabled us to examine the relationship between community composition and the most significant environmental parameters. In order to arrange the

study sites according to their community similarities and through that observe the community response towards the anthropogenic disturbance levels in question, the species data of both bioindicator groups were first used to run an average linkage cluster analysis based on the Bray-Curtis dissimilarity index separately, followed by the same analysis using the combined species data. For the CCA, cluster analysis and community parameter calculations (e.g. rarefied species richness and species diversity) R programming environment (R Core Team 2014) with the 'vegan' (Oksanen 2013b) and 'MASS' (Venables and Ripley 2002) package were used, other statistical analyses were conducted in IBM Statistic SPSS 20.0.

Results

Soil conditions

The mean values for the measured soil characteristics are presented in Table 1. There was no significant difference in the soil characteristics by contamination level; however, significant variation could be detected between individual sampling sites (e.g. lower organic matter content in site N1 compared to the other Kurtna sites). The alkaline air pollution at Kurtna could be observed through mildly elevated soil pH_{H2O} (4.27) compared to Mustoja (3.94), but values of individual samples ranged from acidic to near neutral at Kurtna (Vacht et al. 2014).

Table 1. Measured soil parameters (Vacht et al. 2014) and their values on the two disturbance levels in Kurtna (mild and moderate contamination (and the Mustoja (very low human influence) study area (mean ± *SE* or result of multiple measurements from one mixed sample, true average for pH) together with the range of sulphate content measured in lake water (Terasmaa et al. 2013)

Study area	Very low human influence	Mild contamination	Moderate contamination
Study sites	M1, M2 and M3	J and M	PK, S, A, N1 and N2
Organic matter content (%)	44.6 ± 10.4	35.8 ± 8.6	42.6 ± 6.4
Carbonate content (%)	0.4 ± 0.1	0.9 ± 0.2	1.3 ± 0.3
C (%)	5,7	11.5 ± 0.5	20.5 ± 2.9
N (%)	0,2	0.5 ± 0.0	1.1 ± 0.1
P (mg/kg)	29,1	19.1 ± 1.2	34.6 ± 6.6
K (mg/kg)	42,2	101.0 ± 9.5	121.2 ± 19.4
Ca (mg/kg)	224,4	1616.2 ± 111.1	4731.1 ± 832.3
Mg (mg/kg)	53,2	166.9 ± 18.7	375.4 ± 55.3
pH_{H2O}	3,9	3,8	4,8
SO_4 (mg/L)	-	<20	160-270

Oribatid mite communities and their relation to contamination levels

In total the studied riparian soils held 76 oribatid mite species (Appendix), belonging to 50 genera. The most numerous oribatid species that composed between 5% and 26% of the community of a specific site were *Oppiella nova* (Oudemans 1902), *Conchogneta traegardhi* (Forsslund 1947), *Steganacarus* (*Atropacarus*) *striculus* (Koch 1835) and *Nanhermannia nana* (Nicolet 1855). Kurtna and Mustoja study area shared 71% of the oribatid mite species. Mildly and moderately contaminated sites in Kurtna shared 61% of the oribatid mite species.

The abundance of nine oribatid mite species (Table 2) differed significantly in comparison of the mild and moderate contamination levels. Three of those species (*Tectocepheus velatus* (Michael 1880); *Achipteria coleoptrata* (Linné 1758) and *Minunthozetes semirufus* (C.L. Koch 1841)) were more abundant at the moderate contamination level, where the sulphate concentrations were elevated. *Oppiella (Rhinoppia) hygrophila* (Mahunka 1987) had significantly higher abundance at the mildly contaminate sites. The abundances of all oribatid mite species found from the three contamination levels are presented in the Appendix.

Table 2. Mean abundance (per sample, ± *SE*) or absence (-) of the oribatid mite species that presented a significant difference in their abundance in comparison of the mild and moderate contamination levels in Kurtna with the χ^2 values. Significance levels are reported (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$)

Species	Mild contamination	Moderate contamination	Difference between the two contamination levels (χ^2)
Euphthiracarus cribrarius (Berlese 1904)	0.50 ± 0.40	-	4.945*
Phthiracarus laevigatus (C.L. Koch 1841)	0.70 ± 0.47	-	4.945*
Conchogneta traegardhi (Forsslund 1947)	14.80 ± 5.66	6.00 ± 3.15	5.69*
Oppiella (Rhinoppia) hygrophila (Mahunka 1987)	7.00 ± 3.72	0.29 ± 0.19	12.567***
Oppiella nova (Oudemans 1902)	29.60 ± 11.18	11.75 ± 4.34	5.064*
Tectocepheus velatus (Michael 1880)	0.30 ± 0.30	2.08 ± 0.84	4.686*
Achipteria coleoptrata (Linne 1758)	0.80 ± 0.47	3.04 ± 0.70	5.644*
Minunthozetes semirufus (C.L. Koch 1841)	-	1.79 ± 1.37	4.173*
Pilogalumna crassiclava (Berlese 1914)	0.40 ± 0.31	-	4.945*

Table 3 lists the oribatid mite abundance, observed species richness, values of rarefied species richness, Shannon's diversity and evenness for the three contamination levels and offers a comparison to the diatom community parameters (species richness, diversity and evenness) in Table 4. Comparison of the sites with mild and moderate contamination levels at Kurtna revealed, there were a minor increase in oribatid mite evenness and a small decrease in their diversity with increasing contamination level. Also, the abundance decreased with increasing disturbance level. Mustoja study area had a significantly ($P = 0.049$) higher oribatid mite abundance (182.1 ± 40.6 per samples) than the contaminated Kurtna area on the whole (90.9 ± 15.0 per sample). Oribatid mite species richness did not vary significantly.

Table 3. Oribatid mite community properties in the two disturbance levels in Kurtna (mild and moderate contamination) and as a comparison in Mustoja (very low human influence)

Study area	Very low human influence	Mild contamination	Moderate contamination
Study sites	M1, M2 and M3	J and M	PK, S, A, N1 and N2
Oribatid mite abundance per sample	182.07 ± 40.56	136.80 ± 35.62	71.79 ± 14.02
Oribatid mite abundance per $m^2 \times 10^3$	92.67 ± 20.65	69.63 ± 18.13	36.54 ± 7.13
Estimated oribatid mite species richness †	18	19	19
Observed oribatid mite species richness	39	46	27
Oribatid mite Shannon's diversity	2.59	2.75	2.54
Oribatid mite Shannon's J' evenness	0.71	0.72	0.77

† rarefied species richness (Heck et al. 1975, Oksanen 2013b)

Table 4. Diatom community parameters (Vacht et al. 2014) in the two disturbance levels in Kurtna (mild and moderate contamination) and as a comparison in Mustoja (very low human influence). Observed species richness was not measured for diatoms

Study area	Very low human influence	Mild contamination	Moderate contamination
Study sites	M1, M2 and M3	J and M	PK, S, A, N1 and N2
Estimated diatom species richness †	21	16	18
Diatom Shannon's diversity	2.45	1.70	2.1
Diatom Shannon's J' evenness	0.82	0.68	0.76

† rarefied species richness (Heck et al. 1975, Oksanen 2013b)

Oribatid mite and diatom community similarities between study sites

The site level cluster dendrograms (Figure 2) for diatoms and oribatid mites give an insight into the community similarities between the studied sites. The oribatid mite dendrogram groups together two moderately contaminated sites (N1 and N2) and also separates two other moderately contaminated sites (S and A) from the rest of the Kurtna sites. No clear separation was detected between the Kurtna and Mustoja study areas. Diatom communities, however, form two main clusters, coinciding only on a few occasions with the contamination levels (e.g. M2 and M3 that belong to the Mustoja study area with very low human influence). Even though the Bray-Curtis dissimilarity matrix of the oribatid mite and diatom communities separately did not draw out a classification between the sampling sites in relation to the contamination levels (Figure 2), the combined species data showed a clear distinction between the study sites, and also brought out some differences between the Kurtna study sites (Figure 3). The cluster dendrogram formed three main clusters, separating some of the moderately disturbed study sites in Kurtna (N1 and N2) from the uninfluenced Mustoja sites (M1, M2 an M3) and the rest of the Kurtna sites (J, A, M, S, PK).

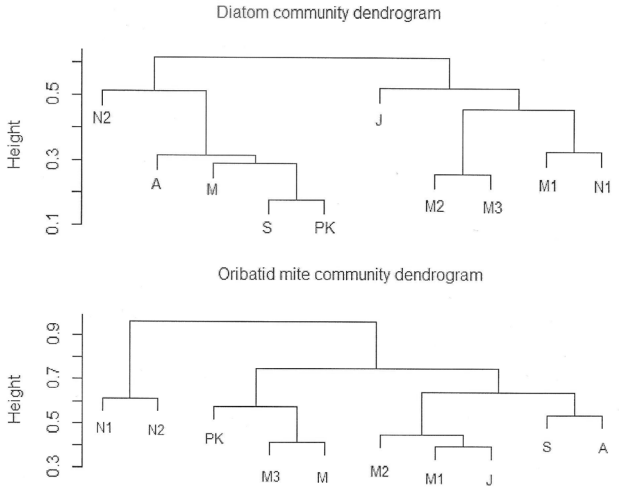

Figure 2. Results of the average linkage cluster analysis based on the Bray-Curtis dissimilarity matrix for diatom and oribatid mite communities as a community dendrogram bringing out the similarities and dissimilarities between the study sites. See Figure 1 for the location of the study sites in the Kurtna and Mustoja kame fields

Community relation to environmental parameters in the Kurtna study area

Along with 18 oribatid mite and 19 diatom species, six environmental variables (sulphate content in lake water $P = 0.101$, soil organic matter $P = 0.031$, total nitrogen $P = 0.009$, phosphorous $P = 0.076$, carbonate $P = 0.577$ and potassium content $P = 0.005$) were included in the CCA of the Kurtna study sites (Figure 4). These six environmental variables explained 30% of the variation in the combined species data while the first axis explained about 43.4% and the second 19.5% of the

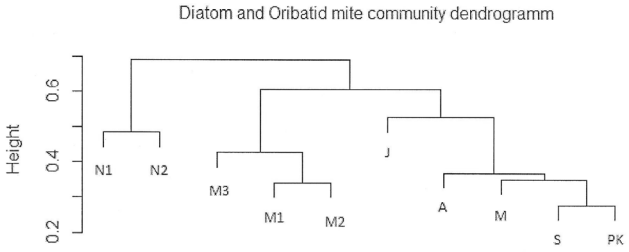

Figure 3. Results of average linkage cluster analysis for combined groups based on the Bray-Curtis distance matrix in the form of a community dendrogram. See Figure 1 for the location of the study sites in the Kurtna and Mustoja kame fields

constrained variability. This analysis indicated that the communities were not strongly correlated with the measured environmental parameters. Most of the moderate but statistically significant correlations were associated with organic matter (e.g. *Chamobates borealis* (Trägårdh 1902): $\rho = 0.344$, $P = 0.043$, *Eupelops torulosus* (Koch 1839): $\rho = 0.354$, $P = 0.037$, *Steganacarus applicatus* (Sellnick 1920): $\rho = 0.495$, $P = 0.003$, *S. striculus:* $\rho = 0.380$, $P = 0.024$, *S. carinatus* (Koch 1841): $\rho = 0.390$, $P = 0.021$). Some phthiracarid mites indicated moderate yet significant ($p < 0.002$) correlations towards changes in soil Ca and Mg content (*S. applicatus* with Ca $\rho = 0.4$ and with Mg $\rho = 0.5$; *S. striculus* with Ca $\rho = 0.5$ and with Mg $\rho = 0.5$). The diatom species were correlated with soil nutrient rather than organic matter content. The significant ($P < 0.05$) correlations to various nutrient concentrations were generally stronger ($\rho > 0.6$) for diatoms than the relationships between oribatid mite species and the environmental parameters.

The oribatid species like *Protoribotritia aberrans* (Markel and Meyer 1959) and *S. applicatus* were less associated with the sulphate contamination. At the same time the diatom species *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst, *Tabellaria fenestrata* (Lyngbye) Kützing, *T. flocculosa* (Roth) Kützing and *Achnanthidium kranzii* (Lange-Bertalot) Round & Bukhtiyarova along with oribatid mite species *O. nova* ($P = 0.024$) and *N. nana* ($P > 0.05$) were abundant when the sulphate content in the lake water was below 20 mg L^{-1} (Terasmaa et al. 2013), while *A. coleoptrata* (Linne 1758) ($P = 0.018$) was more abundant at higher concentrations.

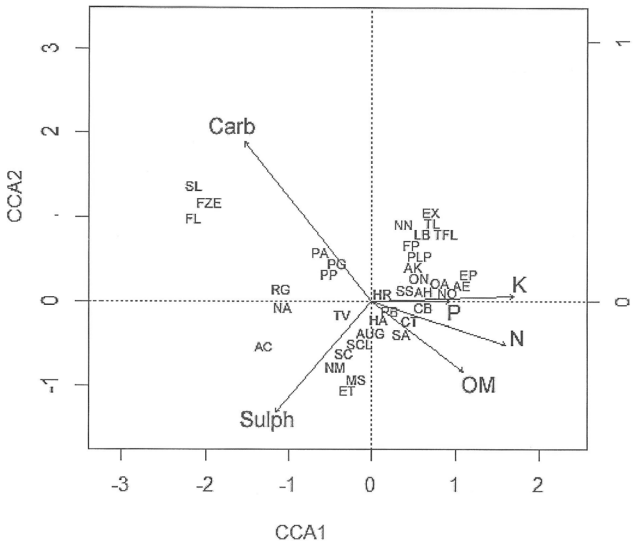

Figure 4. Results of CCA as an ordination plot of combined diatom and oribatid mite data from Kurtna study area with six environmental parameters. Abbreviations: Sulph, sulphate content in lake water; OM, soil organic matter content; N, total nitrogen content in soil; P, content of exchangeable phosphorus in soil; Carb, carbonate content; K, potassium content; diatom species: AE, *Achnanthes exigua*; AH, *A. hungarica*; AK, *Achnanthidium kranzii*; AUG, *Aulacoseira granulata*; EX, *Eunotia exigua*; EP, *E. paludosa*; FL, *Fragilaria leptostauron*; FP, *F. pinnata*; FZE, *F. zeilleri* var. *elliptica*; HA, *Hantzschia amphioxys*; NM, *Navicula mutica*; NO, *N. oblonga*; NA, *Nitzschia amphibia*; PB, *Pinnularia borealis*; PP, *P. perirrorata*; RG, *Rhopalodia gibba*; SL, *Staurosirella leptostauron*; TF, *Tabellaria fenestrata*; TFL, *T. flocculosa*; oribatid mite species: AC, *Achipteria coleoptrata*; CB, *Chamobates borealis*; CT, *Conchogneta traegardhi*; ET, *Eupelops torulosus*; HR, *Hypochthonius rufulus*; LB, *Liochthonius brevis*; MS, *Minunthozetes semirufus*; NN, *Nanhermannia nana*; OA, *Oribatula tibialis*; ON, *Oppiella nova*; PA, *Protoribotritia aberrans*; PG, *Phthiracarus globosus*; PLP, *Platynothrus peltifer*; SS, *Steganacarus striculus*; SA, *Steganacarus applicatus*; SC, *S. carinatus*; SCL, *Scheloribates laevigatus*; TV, *Tectocepheus velatus*

Discussion and conclusions

Even though there was only a slight difference in soil pH_{H_2O} of the two study areas, the contamination gradient is still evident through elevated concentrations of trace metals and other geochemical changes that have been mentioned by previous studies comparing these soils (Kont et al. 1994, 2007, Rooma 1987). It is also possible that today the alkaline air pollution may act as a neutralizing rather than alkalizing factor together with the moderately acidic sulphate rich mining water contamination, therefore, lessening the effects of these disturbances on the soil biota.

The majority of the oribatid mite community consisted of wetland, coniferous forest litter- and soil-dwelling species rather than strictly aquatic species (Luxton 1972, Schatz and Behan-Pelletier 2008, Weigmann 2006). This was surprising considering the fact that, for example, the edges of bog lakes are known to contain numerous aquatic species (Seniczak et al. 2013). Their absence in the studied riparian zones could be linked to e.g. water-level fluctuations in the lakes (Terasmaa et al. 2013) causing periodical drought periods that limit the suitability of these riparian soils for aquatic acarofauna. The diatom community composed of numerous species that are known to live both in aquatic and terrestrial habitats, freshwater species, and also several species whose environmental optimum has not been well researched yet (e.g. *P. lata*) (Vacht et al. 2014).

The percentage of shared species reflects the basic similarities in the habitat conditions of the two areas and the two contamination levels in Kurtna. In both cases there were more shared oribatid mite species (71% and 61% respectively) than diatoms (51% and 54% respectively) (Vacht et al. 2014), which shows that oribatid mite communities were more stable throughout the sampling

sites, thus pointing out their relative robustness as bioindicators (e.g. Gulvik 2007, Behan-Pelletier 1999). This difference could also be caused by the absence of strictly aquatic oribatid mite species compared to diatom communities that contained also species commonly found in freshwater habitats (Vacht et al. 2014). The different response of these two bioindicator groups can also be noticed in the percentage of shared species: There were more shared diatom species in the two contamination levels in Kurtna than in the two study areas (Vacht et al. 2014), while for oribatid mites the situation was reversed. While for diatoms the change in shared species was relatively minor (Vacht et al. 2014), the percentage of shared species for oribatid mites changed considerably. This indicates that not only was the change in shared species of the two groups reversed regarding the contamination gradient, but the response was also different in its extent.

Due to the nature of diatom extraction methodology, the abundance of diatoms was not evaluated and only the oribatid mites were investigated from this aspect. The results showed a clear decrease in oribatid mite abundance with increasing contamination level, the abundance at the moderate contamination level differed roughly two-fold compared to the mild contamination level. On the whole, the range of oribatid mite abundance was lower than some studies from Poland registered in fairly undisturbed forest floor (Skubała and Marzec 201, Lósková et al. 2013) but reaching notably higher abundances than e.g. industrial dumps (Skubała 1995). Their abundance in the moderately contaminated sites can be compared to Slovakian sites affected by e.g. clear-cutting or wildfire (Lósková et al. 2013). Therefore, the abundance of oribatid mites can be considered notably low only on the site with moderate contamination.

The oribatid mite species richness in the mildly contaminated Kurtna sites and the Mustoja study area was similar to the number of species found, e.g. a pine forests affected by air pollution in Poland (Seniczak et al. 1998). The influence of contamination effects on oribatid mites cannot be interpreted solely by the observed species richness because overall the number of oribatid mite species can be considered low compared to other studies (e.g. Horwood and Butt 2000, Luptáčik 2012). Diatom communities had clearly higher estimated species richness at the fairly undisturbed Mustoja study area but the two contamination levels in Kurtna showed no significant difference (Vacht et al. 2014). Overall, the species richness of neither groups appeared to be strongly influenced by the contamination levels (Vacht et al. 2014). The change in diversity and evenness with increasing contamination level in Kurtna for the two bioindicator groups showed minor and sometimes contradictory results, implying that the way these indicator communities are affected as a whole was different. This means the two bioindicators show some complimentary qualities. The low magnitude of change in community diversity was expected, explained by previous studies implying changes rather in species composition than diversity due to environmental changes in riparian areas (Naiman and Déchamps 1997). Considering that the riparian zones are often biologically diverse (e.g. Naiman and Décamps 1997, Seniczak et al. 2006), and species rich (Malanson 1993, Naiman et al. 1993), the abundance and relatively low species richness in the moderately contaminated sites could be linked to the anthropogenic disturbances (Naiman and Décamps 1997). However, the extent and variability of these changes implies that the studied disturbances had a weak effect on the communities.

Nevertheless, many eurytopic oribatid mite species, such as *A. coleoptrata* (Linnaeus 1758) and *T. velatus* (Michael 1880), had higher abundance at the highest contamination level (Luxton 1979), which could be considered as a form of bioindication. *T. velatus*, for example, is known for elevated abundance under the effects of various anthropogenic disturbances (e.g. Gulvik 2007, Skubała and Zaleski 2012, Ivan and Vasiliu 2009). Many of the oribatid mite species found are known for their abilities to accumulate trace metals (e.g. *T. velatus*) (Skubała and Zaleski 2012), which could also explain their abundance at Kurtna. *A. coleoptrata* is not as well known for its indicativeness of strong contamination, it has rather been found from mildly or moderately affected sites (Skubała and Zaleski 2012, Caruso and Migliorini 2006). The species that have previously been considered sensitive to air pollution (e.g. Seniczak and Dabrowski 1995, Kehl and Weigmann 1992) e.g. *Carabodes labyrinthicus* (Michael 1879), *Trichoribates trimculatus* (Koch 1835) are represented in too low numbers in order to draw specific conclusions on their indicativeness to alkaline air pollution in the Kurtna study area. The species indicating most significantly the decrease in contamination levels with its increasing abundance was *O. hygrophila* which can for this reason be considered as a potential indicator species of anthropogenic disturbances. However, there are no specific linkages connecting the species to changes in sulphate concentrations or alkaline air pollution. In addition, there is currently very little information on its environmental preferences in general making it impossible to draw further conclusions.

The diatom communities at the highest contamination level, and the Kurtna study area as a whole, showed high levels of ubiquitous diatom flora (Vacht et al. 2014). Some species (e.g. *P. lata*) decreased in their abundance with increasing contamination level (Vacht et al. 2014) but in most cases only a moderate shift was observable. Overall, diatom communities were characterized by their

high variability between study sites and single samples (Vacht et al. 2014). This implies that using only the riparian diatom flora, with very few species potentially suitable for bioindication, as an indicator of sulphate-rich mining waters would give insufficient results.

Oribatid mite communities in riparian areas are generally extensively studies; and the existing research (e.g. Seniczak et al. 2013, Seniczak 2011) does not mention linkages between the communities nor the specific species encountered in this study and sulphate concentrations. Previous studies have reported that diatom communities respond to changes in the sulphate content (e.g. De la Rey et al. 2004), but the species-specific indicativeness of these observations is less studied (e.g. Luis et al. 2012). Our earlier detailed analysis of the diatom community structure and parameters in relation to lake water sulphate concentrations (Vacht et al. 2014) noted *R. gibba* (Ehrenberg) Müller and *F. zeilleri* var. *elliptica* (Gasse) as species that react positively to elevated sulphate concentration levels. Some research suggests that sulphates may influence the diatom community through nutrient uptake (Saros and Fritz 2000).

The cluster analysis showed clearly that based on the community structure and through that the similarities of specific sites; the differentiations between contamination levels were less obvious when viewed solely through diatom or oribatid mite communities than when observed through the combined use of the two indicator groups. However, even then, the difference was only clear enough to separate the communities on two study areas, and one is less clear between the two contamination levels in Kurtna. Therefore, it is likely that the sulphate concentrations are not the primary environmental factors controlling the oribatid mite and diatom communities in these riparian soils.

Neither of the studied bioindicators is flawless, indicating immaculately the sulphate concentrations nor the three categorical contamination levels that were investigated. The high variability of these communities, presence of species whose species-specific indication properties are not yet well understood and the unexpectedly weak influence of the measured environmental parameters on the community as a whole, present difficulties in interpreting the two indicator groups. While some information exists on aquatic Oribatida indicating changes in lake water (Solhøy and Solhøy 2000), there is a knowledge gap in understanding the effects of water contamination on mainly terrestrial oribatid communities. Based on previous knowledge and data gained from this study, it is currently possible to rather observe a shift in oribatid communities due to the difference between the two study areas, that were differentiable through the long-term effects of alkaline air pollution also rich in trace metals (Kont et al. 1994, 2007, Rooma 1987) than due to the change in sulphate concentrations. The two bioindicator groups have been used in numerous occasion as multi-proxy bioindicators in paleoecologically, especially paleolimnological, studies (e.g. Luoto 2009, Solhøy and Solhøy 2000). However, in these studies, diatoms are usually the main indicating agents, while the use of species-specific bioindicator qualities of oribatid mites are often overlooked because of their low abundance (Solhøy and Solhøy 2000). In soil ecosystem the situation is reversed, the abundance of oribatid mites exceeding that of diatoms. This study confirmed that in riparian areas the two groups are presented in sufficient numbers creating a suitable environment for studying their combined bioindication abilities. Even though interface environments such as riparian zones are particularly sensitive to environmental change (Malanson 1993, Naiman and Déchamps 1997), it also true that the riparian zones are challenging ecosystems for studying soil bioindicators in general due to accelerated decomposition rates and the fragile balance between nutrient buffering (Naiman and Déchamps 1997) and rapid nutrient release (Edmonds 1980) adding to the possible changes in environmental parameters. Therefore, even though these ecosystems are suitable for the combined use of oribatid mites and diatoms, riparian zone soils also present a series of challenges in interpreting the effects of anthropogenic disturbances on the community patterns. This could explain the reason why the differentiation between the disturbances from various origins remained weaker than originally anticipated.

In conclusion, it can be indicated that oribatid communities express significant differences between the mild and moderate contamination levels through changes in their abundance and some species-specific variation. While oribatid communities contained mostly litter- and soil-dwelling species rather than strictly aquatic species, diatom communities contained both aquatic and aerial species. This provided new insights into the diversity of diatoms and oribatid mites in riparian zone forest soils in Estonia, proposing also some species from both groups as potential indicators of sulphate-rich mining water contamination. The two bioindicator groups reacted differently to the studied disturbances meaning that the groups carry complementary information. Based on the cluster analysis and comparative analyses of the changes in community parameters and structure in regards to contamination levels the integrated use of diatoms and oribatid mites provided more accurate bioindication that the two groups separately. Their combined use enabled the two study areas to be roughly separated, although based on the community structure of combined species data no clear separation could be made between the Kurtna sites with lower sulphate concentrations in lake water and the elevated ones. Even

though riparian zone soils are suitable study systems for the combined use of diatoms and oribatid mites, each carrying their own strengths as bioindicators, these areas offer challenges for interpreting the effects of long-term but mild anthropogenic disturbances on these groups. More specifically, at the current magnitude the disturbances may not have been sufficiently strong to bring out the full indicative potential of these indicator groups.

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Appendix. Mean abundances per sample (± SE, 196 cm^3, 10 cm depth, sampled in September 2012 and 2013) or absences (-) of oribatid mites at the very low human influence level in Mustoja (sites M1, M2 and M3, n = 30), mildly contaminated sites in Kurtna (J and M, n = 20) and moderately contaminated sites in Kurtna (PK, S, A, N1 and N2, n = 50)

Species	Very low human influence	Mild contamination	Moderate contamination
Brachychthonius berlesei (Willmann, 1928)	0.13 ± 0.13	-	0.08 ± 0.06
Liochthonius brevis (Michael, 1888)	-	4.7 ± 3.89	0.38 ± 0.29
Liochthonius furcillatus (Willmann, 1942)	-	0.50 ± 0.5	-
Hypochthonius rufulus (C.L. Koch, 1835)	0.20 ± 0.10	9.10 ± 5.18	3.58 ± 1.17
Eniochthonius minutissimus (Berlese, 1903)	-	-	0.08 ± 0.06
Eulohmannia ribagai (Berlese, 1910)	-	0.30 ± 0.30	0.04 ± 0.04
Phthiracarus ferrugineus (C.L. Koch, 1841)	0.47 ± 0.19	0.90 ± 0.61	1.00 ± 0.35
Phthiracarus globosus (C.L. Koch, 1841)	0.73 ± 0.28	3.20 ± 1.41	1.92 ± 0.44
Phthiracarus laevigatus (C.L. Koch, 1844)	0.33 ± 0.33	0.70 ± 0.47	-
Steganacarus applicatus (Sellnick, 1920)	-	0.80 ± 0.44	1.13 ± 0.40
Steganacarus (Tropacarus) carinatus (C.L. Koch, 1841)	7.20 ± 2.03	3.60 ± 2.63	6.29 ± 2.12
Steganacarus magnus (Nicolet, 1855)	0.27 ± 0.15	0.70 ± 0.52	0.13 ± 0.07
Steganacarus (Atropacarus) striculus (C.L. Koch, 1835)	11.47 ± 7.87	12.70 ± 4.29	6.58 ± 1.90
Euphthiracarus cribrarius (Berlese, 1904)	0.07 ± 0.07	0.50 ± 0.40	-
Microtritia minima (Berlese, 1904)	5.87 ± 2.81	0.10 ± 0.10	-
Rhysotritia ardua (C.L. Koch, 1841)	-	0.20 ± 0.13	0.04 ± 0.04
Protoribotritia aberrans (Märkel & Meyer 1959)	1.40 ± 0.52	0.50 ± 0.17	2.13 ± 0.98
Platynothrus peltifer (C.L. Koch, 1839)	5.60 ± 2.76	4.40 ± 2.67	2.46 ± 0.53
Heminothrus longisetosus (Willmann, 1925)	-	-	0.08 ± 0.06
Nothrus borussicus (Sellnick, 1928)	1.87 ± 0.79	-	0.04 ± 0.04
Nothrus palustris (C.L. Koch, 1839)	1.73 ± 0.80	1.10 ± 0.64	0.13 ± 0.09
Nothrus pratensis (Sellnick, 1928)	0.20 ± 0.10	0.30 ± 0.21	0.46 ± 0.23
Nothrus silvestris (Nicolet, 1855)	10.67 ± 3.89	1.80 ± 1.04	0.38 ± 0.16
Malaconothrus monodactylus (Michael, 1888)	0.07 ± 0.07	0.40 ± 0.40	0.04 ± 0.04
Nanhermannia nana (Nicolet, 1855)	23.60 ± 14.12	20.60 ± 11.41	2.71 ± 1.05
Hermannia gibba (C.L. Koch, 1839)	-	-	0.04 ± 0.04
Hermanniella dolosa (Grandjean, 1931)	0.40 ± 0.19	0.30 ± 0.21	0.13 ± 0.09
Metabelba spp (Grandjean, 1936)		1.33 ± 0.33	2.00 ± 0.41
Belba spp (von Heyden, 1926)	-	1.00 ± 0.00	-
Damaeus gracilipes (Kulczynski, 1902)	0.13 ± 0.13	-	0.04 ± 0.04
Spatiodamaeus verticillipes (Nicolet, 1855)	0.53 ± 0.32	0.20 ± 0.20	0.08 ± 0.06
Cepheus cepheiformis (Nicolet, 1855)	-	-	0.21 ± 0.17
Caleremaeus monilipes (Michael, 1882)	0.40 ± 0.21	-	0.13 ± 0.13
Liacarus spp (Michael, 1898)	1.00 ± 0.00	-	-
Adoristes ovatus (C.L. Koch, 1839)	0.20 ± 0.20	0.3 ± 0.15	0.25 ± 0.14

Appendix (*continued*)

Species	Very low human influence	Mild contamination	Moderate contamination
Xenillus tegeocranus (Hermann, 1804)	-	0.20 ± 0.13	-
Astegistes pilosus (C.L. Koch, 1840)	1.07 ± 0.51	-	0.04 ± 0.04
Ceratoppia spp (Berlese, 1908)	1.00 ± 0.00	-	-
Gustavia microcephala (Nicolet, 1855)	0.07 ± 0.07	-	0.08 ± 0.06
Carabodes areolatus (Berlese, 1916)	-	-	0.25 ± 0.14
Carabodes labyrinthicus (Michael,1879)	0.47 ± 0.29	-	0.04 ± 0.04
Carabodes rugosior (Berlese, 1916)	12.27 ± 5.89	-	0.04 ± 0.04
Carabodes willmanni (Bernini, 1975)	1.07 ± 0.75	-	0.08 ± 0.08
Conchogneta traegardhi (Forsslund, 1947)	8.47 ± 8.33	14.80 ± 5.66	6.00 ± 3.15
Dissorhina ornata (Oudemans, 1900)	-	-	0.04 ± 0.04
Oppiella (Rhinoppia) hygrophila (Mahunka, 1987)	42.87 ± 12.83	7.00 ± 3.72	0.29 ± 0.19
Oppiella (Moritzoppia) translamellata (Willmann, 1923)	0.07 ± 0.07	2.50 ± 1.13	3.17 ± 1.27
Oppiella nova (Oudemans, 1902)	3.00 ± 1.46	29.60 ± 11.18	11.75 ± 4.34
Tectocepheus minor (Berlese, 1903)	6.33 ± 2.84	0.20 ± 0.13	1.08 ± 0.51
Tectocepheus velatus velatus (Michael, 1880)	0.73 ± 0.37	0.30 ± 0.30	2.08 ± 0.84
Eupelops acromios (Hermann, 1804)	-	0.10 ± 0.10	0.08 ± 0.06
Eupelops hirtus (Berlese, 1916)	-	-	0.04 ± 0.04
Eupelops tardus (C.L. Koch, 1835)	0.13 ± 0.09	0.10 ± 0.10	0.13 ± 0.09
Eupelops torulosus (C.L. Koch, 1839)	0.07 ± 0.07	0.50 ± 0.17	2.08 ± 1.49
Achipteria coleoptrata (Linné, 1758)	0.13 ± 0.13	0.80 ± 0.47	3.04 ± 0.70
Oribatula tibialis (Nicolet, 1855)	1.00 ± 0.50	0.10 ± 0.10	0.13 ± 0.13
Liebstadia pannonica (Willmann, 1951)	0.13 ± 0.09	0.40 ± 0.40	0.13 ± 0.13
Scheloribates laevigatus (C.L. Koch, 1835)	0.53 ± 0.24	0.90 ± 0.64	0.96 ± 0.49
Scheloribates latipes (C.L. Koch, 1844)	3.87 ± 1.15	0.10 ± 0.10	0.29 ± 0.15
Chamobates borealis (Trägårdh, 1902)	1.73 ± 0.63	7.50 ± 5.00	3.75 ± 1.80
Chamobates cuspidatus (Michael, 1884)	2.47 ± 1.15	-	0.04 ± 0.04
Chamobates pusillus (Berlese, 1895)	-	0.10 ± 0.10	0.13 ± 0.07
Chamobates voigtsi (Oudemans, 1902)	17.93 ± 9.16	0.20 ± 0.20	0.79 ± 0.51
Ceratozetes mediocris (Berlese, 1908)	0.20 ± 0.20	0.20 ± 0.20	0.50 ± 0.27
Ceratozetes parvulus (Sellnick, 1922)	0.07 ± 0.07	0.10 ± 0.10	0.04 ± 0.04
Trichoribates trimaculatus (C.L. Koch, 1835)	-	-	0.04 ± 0.04
Euzetes globulus (Nicolet, 1855)	0.53 ± 0.17	0.60 ± 0.43	0.83 ± 0.50
Minunthozetes semirufus (C.L. Koch, 1841)	0.13 ± 0.13	-	1.79 ± 1.37
Mycobates bicornis (Strenzke, 1954)	0.40 ± 0.40	0.40 ± 0.22	0.54 ± 0.19
Punctoribates hexagonus (Berlese, 1908)	0.07 ± 0.07	1.10 ± 0.89	0.42 ± 0.18
Galumna flagellata (Willmann, 1925)	0.27 ± 0.12	-	0.04 ± 0.04
Galumna lanceata (Oudemans, 1900)	0.93 ± 0.45	0.40 ± 0.40	0.08 ± 0.06
Galumna obvia (Berlese, 1914)	-	0.20 ± 0.20	0.25 ± 0.17
Pergalumna formicaria (Berlese, 1914)	0.27 ± 0.21	-	0.04 ± 0.04
Pergalumna nervosa (Berlese, 1914)	0.87 ± 0.27	0.10 ± 0.10	0.17 ± 0.10
Pilogalumna crassiclava (Berlese, 1914)	0.40 ± 0.29	0.40 ± 0.31	-