## Variability of riparian soil diatom communities and their potential as indicators of anthropogenic disturbances

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Abstract. Riparian soils are affected by both natural and anthropogenic disturbances occurring in the water bodies and on the catchment area. These riparian areas are also rich in microhabitats and therefore host various soil biota, including diatoms. Diatoms are known for their bioindication abilities in water and could potentially be used in that context in the riparian zone. Therefore the possibility of riparian soil diatoms acting as indicators of both terrestrial and aquatic disturbances is worth discussion. We analysed diatom community structure and their variability between different study areas and sites. We also quantified diatom species diversity and richness and evenness of the riparian topsoils. Possible effects of various anthropogenic disturbances on diatom communities, alkaline air pollution, and the effects of mining waters pumped into the area were studied in northeastern Estonia. These results were compared with results from an area with low human influence in south-eastern Estonia. Additionally, we evaluated the potential of diatoms as indicators of various anthropogenic disturbance levels and a water contamination gradient based on sulphate concentrations. Community parameters, including species richness, diversity, and evenness, indicated some differences between the studied communities both when the separate study sites and distinguishable anthropogenic disturbance levels were compared. Diatom assemblages also showed moderate variability between the study sites, which could be influenced by variable moisture conditions, variable organic matter content, and the trophic level of the water body. Despite the variable levels of human influence the two compared areas shared about 51.4% of the species. Our findings show that the diatom community composition of riparian soils could potentially indicate anthropogenic disturbance levels, especially through the abundance, absence, or presence of specific species (e.g. Hantzschia amphioxys, Fragilaria zeilleri var. elliptica, Pinnularia lata).

Key words: diatoms, soil, riparian zone, bioindicators, human influence, disturbances.

## **INTRODUCTION**

Soil is known for its high but still fairly unknown biodiversity. The decline of soil biodiversity due to anthropogenic influences has been recognized as an important ecological problem (e.g. Fierer et al., 2009). However, only some groups of

organisms (e.g. earthworms) in soil are well understood, leaving a vast amount of biota and a number of habitats unresearched. Because the functions and services of the terrestrial ecosystem depend on soils and soil biodiversity, it is important to identify bioindicators for both natural and anthropogenic environmental trends and changes (Havlicek, 2012).

A number of different bioindicators (e.g. earthworms, microarthropods) are used to evaluate soil conditions (e.g. Ivan and Vasiliu, 2009), each with a different response to environmental changes. For example, in Estonia earthworms have been studied in flooded coastal grasslands, suggesting that species react differently to variable moisture conditions (Ivask et al., 2007). However, there are still many soil organisms with indicator properties that are understudied. An example of these is soil algae, which are among the first organisms to colonize soil (Starks et al., 1981). Among them are diatoms, eukaryotic single-celled algae, whose spatiotemporal distribution is constantly influenced by a combination of human activities and natural processes.

Although mostly known for their short response times to environmental changes and indicator abilities in water (e.g. Kobayasi and Mayama, 1982; Eloranta and Soininen, 2002; de la Rey et al., 2004; Levkov et al., 2007; Beyene et al., 2014), diatoms can also be found in soils and are considered to be potentially good environmental indicators of terrestrial ecosystem properties (van Kerckvoorde et al., 2000; Berard et al., 2004; Van de Vijver et al., 2008; Kabirov and Gaisina, 2009; Moravcová et al., 2009; Heger et al., 2012). For example, soil diatoms have been studied in relation to agricultural activities (Heger et al., 2012), oil pollution (Dorokhova, 2007), and in polar areas in the context of vegetation type (van Kerckvoorde et al., 2000; Van de Vijver et al., 2008) and animal perturbation (Moravcová et al., 2009).

Riparian zones are strongly affected by both aquatic and terrestrial environmental changes. Water-level fluctuations are often mediators of these changes and serve as a major source of nutrients, sediments, and water to the riparian soils (Grobbelaar, 1983; Weilhoefer and Pan, 2007). Therefore, not only do the riparian zones impact the development of the aquatic ecosystem and its trophic status, but the water body may also alter the physical and chemical parameters of the riparian habitat (e.g. floodwaters) (Weilhoefer and Pan, 2007). Through various alterations in their habitat, the riparian biota, including soil diatoms, are influenced by changes in the water body and on the catchment area. Understanding processes in riparian zones is a great challenge due to their variable environmental conditions. Therefore, exploring diatom variability in riparian areas can potentially enable us to explore the influence of anthropogenic disturbances on diatom communities.

Based on previous studies from aquatic (e.g. Dickman, 1998; Gudmundsdottir et al., 2013) and terrestrial environments (e.g. Kobayasi and Mayama, 1982; Zancan et al., 2006) together with earlier studies from Estonia where diatoms were studied on moist road-side soils (Vacht, 2012, 2014), the following hypotheses were formed: (1) the properties and structure of the riparian soil diatom community vary depending on the study area and site, soil and water body characteristics, and anthropogenic disturbances, but a partially common diatom community is maintained;

and (2) diatom species richness and diversity decrease and community evenness increases with increasing human influence. Based on these hypotheses, the following goals were set: (1) to analyse diatom community parameters and structure taking into account both the variability of the sampling sites and the multiple types of anthropogenic disturbances they are subject to; (2) to discuss the potential of using riparian soil diatoms as bioindicators of anthropogenic disturbances.

## STUDY AREAS

The current study focuses on analysing the variability of riparian diatom communities in one of the most anthropogenically disturbed natural areas in Estonia – the Kurtna Kame Field (59°16' N, 27°34' E) located in north-eastern Estonia, which is compared with an area unaffected by intensive human influence located in south-eastern Estonia on the Mustoja Kame Field (57°53' N, 27°39' E) (Fig. 1). Considering genesis and structure, the Kurtna and Mustoja Kame Fields are the most similar kame fields among 50 Estonian kame fields (Kont et al., 1994).

In the Kurtna area eight sites (N1, N2, A, S, PK, K, M, and J) and at Mustoja three sites (M1–M3) were selected. The existence of previous studies on the nearby water bodies was taken into account in the selection of the study sites within the study areas. All of the study sites were located on moist soils with a peaty (P-horizon) or partially decomposed organic layer (H-horizon) exposed to water for prolonged periods each year, either from floodwaters or water seepage from the lake by capillary rise or both. The study sites experience only brief periods of dryness throughout the year.

These kame fields are generally characterized by acidic and nutrient poor podzols. However, in the riparian soils the soil type ranges rather from gleyic podzols to histosols. While the predominant vegetation type on both kame fields



Fig. 1. Location of the study areas: K – Kurtna Kame Field, M – Mustoja Kame Field.

is pine (*Pinus sylvestris* L.) forest, the riparian vegetation varied between sampling sites, reflecting variable moisture conditions containing for example *Polytrichum commune* L., *Sphagnum* spp., *Equisetum* spp., and *Alnus incana* L. The average yearly precipitation at Kurtna is about 696 mm and at Mustoja 634 mm; the average yearly temperature is 4.7 °C and 5.7 °C, respectively (Estonian Environment Agency, 2014).

## Anthropogenic disturbances at the Kurtna Kame Field

The Kurtna study area can be described as a transitional zone between an industrialized mining and processing region (oil shale and peat) and a sparsely populated natural periphery (Punning et al., 2007). An increase of the human influence in the area began in the 1950s. In the 1980s thermal power plants erected in the area emitted annually 200 000 tonnes of fly ash to the atmosphere (Punning et al., 1989), causing geochemical changes in the lakes (e.g. Punning et al., 1997; Marzecová et al., 2011) and soils (Rooma, 1987; Kont et al., 1994). With the alkaline deposition harmful trace substances such as heavy metals and sulphur also accumulated (Kont et al., 1994). Even though the amount of air pollution has decreased, enabling the ecosystem to recover, the effects of the pollution can still be observed (Kont et al., 1994, 2007).

In the 1970s, alkaline mining waters with a high sulphate content began to be pumped into the Raudi Channel (Vesiloo, 1987) flowing through a number of lakes. In these lakes the average yearly increase in the sulphate concentration from 1937 to 2012 ranged from 0.28 to 3 mg L<sup>-1</sup>, exceeding in some of the lakes 200 mg L<sup>-1</sup> (Terasmaa et al., 2013). All our study sites at Kurtna (N1, N2, A, S, PK, K, M, and J) (Fig. 2) were also exposed to fly-ash fallout. Two of the Kurtna



**Fig. 2.** Location of the study sites in the Kurtna Kame Field. The abbreviations stand for the lake names: N1 – Lake Nõmmejärv (site 1), N2 – Lake Nõmmejärv (site 2), S – Lake Särgjärv, A – Lake Ahvenjärv, PK – Lake Peen-Kirjakjärv, K – Lake Kirjakjärv (affected by mining waters and air pollution), M – Lake Martiska, J – Lake Jaala (affected by air pollution).

study sites (M and J) have not been directly affected by the mining waters and are therefore characterized by a lower anthropogenic disturbance level (Disturbance 1) compared to the other six sites there (N1, N2, A, S, PK, and K) (Disturbance 2). Additionally, a gradient based on sulphate (SO<sub>4</sub>) concentrations in the water (Terasmaa et al., 2013) was used as a measure of disturbance levels in the lakes.

## **METHODS**

## **Diatom sampling**

Samples were collected in September 2012 from three sites at Mustoja and eight sites at Kurtna from the riparian zone in five repetitions parallel to the lake or stream shore, approximately 1-2 m from the water body. In order to get an overview of diatom variability also within the study site, the different microhabitats and the apparent impact of water on vegetation characteristics were taken into account while sampling. The locations of the Kurtna study sites are indicated in Fig. 2. For diatom analysis, soil samples were taken from the top 4 cm of soil with a soil auger (Ø 5 cm) and kept at 4°C until sample preparation.

## Diatom sample preparation and identification

Each sample was mixed thoroughly and  $2 \text{ cm}^3$  of soil was extracted. The soil was treated with 10% HCl and heated for 2 min to remove the carbonates, then 30%  $H_2O_2$  was added to the solution and it was heated until the remainder of organic matter was removed. The samples were decanted and centrifuged to remove other unnecessary components. A single drop of solution was transferred to the cover glass, allowed to dry, and then fixed with Naphrax. Slides were examined under  $600-1000 \times \text{magnification}$  (using immersion oil nd = 1.516) on an Olympus BX51 microscope. From each sample 300-500 valves were counted along random transects. Damaged valves were counted as a separate individual when more than half of the valve was intact. Because the diatom taxonomy is subject to continuous transfers and changes, the 'old' nomenclature (Krammer and Lange-Bertalot, 1988–1991, 1999–2004) supplemented with additional taxonomic materials (e.g. Round and Bukhtiyarova, 1996; Souffreau et al., 2013) was used for identification. Diatoms were identified to species level and when this was impossible due to damage, to genus level (Krammer and Lange-Bertalot, 1988– 1991, 1999–2004; Lange-Bertalot and Metzeltin, 1996; Lange-Bertalot et al., 2011).

## Soil sampling and analysis

Although it is known from previous studies (Kont et al., 2007) that the soils in these areas are similar, some additional analyses were made to describe the riparian soils. Soil samples were collected with a soil auger ( $\emptyset$  5 cm) and kept at 4 °C until analysis. Water (W), organic matter (OM), and carbonate content (Carbonates)

were measured from the soil samples using standard methods (Heiri et al., 2001). Additionally, soil  $pH_{H2O}$  (pH) was measured using WTW pH Electrode SenTix41. An average sample from each study site was analysed for total nitrogen (N), carbon (C), and phosphorus (P) using AL extraction. For potassium (K), calcium (Ca), and magnesium (Mg) AL extraction with the MP-AES was used. The samples were analysed in the soil laboratories of the Estonian University of Life Sciences.

## **Data processing**

In order to characterize diatom diversity, we calculated species richness using rarefaction analysis in R (Heck et al., 1975; Oksanen et al., 2013), Shannon's diversity (H'), and evenness (J'). The calculations of Shannon's diversity and evenness were based on diatom counts. The preliminary analysis of the diatom communities and environmental parameters was conducted using various descriptive and generalizing statistical methods. In order to test for differences in soil properties (W, OM, Carbonates, etc.) and main community properties (richness, Shannon's diversity, and evenness) between the study areas and disturbance levels, T-test was used. Differences in soil and community parameters between the study sites and trophic levels (dyseutrophic, soft-water eutrophic, and hard-water mesotrophic) were tested using one-way analysis of variance (ANOVA). To clarify which sites/trophic levels differed in terms of soil and/or community properties, ANOVA was followed by post hoc tests – LSD in case of equal variances or Tamhane's T2 in case of unequal variances. Correlation analysis (Spearman correlation  $\rho$ ) was used to determine the strength of the associations between the variables. These tests were conducted in IBM SPSS 20.0.

Canonical Correspondence Analysis (CCA) (Ter Braak, 1986) was used to examine the associations between diatom community composition, environmental variables (C/N, W, OM, Mg, P, pH, K, Ca), and disturbance levels (disturbance groups 1 and 2 and SO<sub>4</sub> concentrations). At Mustoja the environmental variables showed little variation between the study sites and considering the small number of sites, CCA was run only with data from the Kurtna study area. To decrease the influence of rare species on the CCA outcome, only species with an excess of 0.1% of the total species abundance were included in the analysis (39 species). To find the combination of explanatory variables that best explains the variation in diatom data, we used a forward selection procedure with Monte Carlo permutation tests (999 permutations) until all significant variables were included in the CCA model. CCA was carried out using R programming environment (R Core Team, 2014) and the package 'vegan' (Oksanen et al., 2013).

## RESULTS

#### Soil properties

Soil moisture (W), organic matter (OM), and carbonate content (Carbonates) showed significant (p < 0.05) differences between study sites (Table 1) (W F = 3.9,

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**Table 1.** Results of soil analysis (mean  $\pm$  SE or single measured value from an average sample). SeeFig. 2 for the location of the study sites in the Kurtna Kame Field; M1–M3 – study sites in theMustoja Kame Field

Site	Water content, %	Organic matter content, %	Carbonate content, %	C/N ratio	P, mg kg <sup>-1</sup>	K, mg kg <sup>-1</sup>	Ca, mg kg <sup>-1</sup>	Mg, mg kg <sup>-1</sup>	pH <sub>H2O</sub>
N1	$26.90 \pm 8.3$	$7.50 \pm 2.9$	2.16±1.4	15.70	11.51	23.23	770.73	83.72	5.73
N2	$36.32\!\pm\!6.3$	$19.47 \pm 5.1$	$0.73 \pm 0.1$	15.25	8.06	15.99	433.46	65.81	6.06
А	$55.43 \pm 5.1$	$46.08 \pm 9.5$	$1.21\!\pm\!0.3$	22.57	25.93	213.15	11 358.16	599.89	4.47
S	$57.10 \pm 6.4$	$65.24 \pm 13.1$	$1.13\pm0.2$	16.64	28.15	93.09	4 167.45	338.06	4.64
PK	$73.24 \pm 1.4$	$71.95 \pm 3.3$	$3.32 \pm 0.4$	20.57	94.11	239.39	6 066.08	727.46	5.20
Κ	$77.06 \pm 1.3$	$84.79 \pm 1.3$	$1.85 \pm 0.7$	24.05	68.57	243.97	7 962.39	1 391.03	5.05
J	$62.57 {\pm} 8.9$	$48.79 \pm 13.4$	$1.31\!\pm\!0.2$	22.07	20.55	129.40	1 949.40	222.90	5.19
М	$39.18 \pm 7.4$	$22.84 \pm 9.1$	$0.54 \pm 0.2$	30.49	16.05	72.50	1 283.00	110.90	3.52
M1-M3	$46.13\pm7.5$	$44.6 \pm 10.4$	$0.42\pm0.1$	35.56	29.05	42.15	224.43	53.24	3.94

p < 0.001; OM F = 4.7, p < 0.001; Carbonates F = 4.2, p < 0.001). The exceptionally low yet highly variable organic matter content at the N1 site (mean  $7.4\pm6.5\%$ ) and maximum carbonate content at the PK site  $(3.3\pm0.4\%)$  affected the variability of soil properties between the sites. Carbonate content showed significant (p < 0.05) differences also between the study areas (t = 5.0, p < 0.001). However, soil moisture (Kurtna  $51.7\pm3.1$ , Mustoja  $46.1\pm7.5$ , t = 0.7,  $p \ge 0.05$ ) and organic matter content (Kurtna  $43.2\pm4.6$ , Mustoja  $44.6\pm10.4$ , t = -0.1,  $p \ge 0.05$ ) did not differ between the two study areas. The pH<sub>H2O</sub> was higher at the Kurtna study area (4.27) than at Mustoja (3.94) but for individual samples it ranged from acidic to near neutral in both study areas and the difference was not significant.

The C/N ratio was relatively high: at the Kurtna study sites it ranged from 15.25 to 30.49 and was 35.56 at Mustoja (Table 1). The contents of available phosphorus, potassium, calcium, and magnesium varied, indicating elevated values at some sites at Kurtna (e.g. PK and K). The calcium and magnesium contents were significantly correlated with the overall carbonate content ( $\rho > 0.6$ , p < 0.05). Also the organic matter content, moisture content, and the contents of many of the soil nutrients there were significantly intercorrelated, especially organic matter and soil moisture content ( $\rho = 0.95$ , p < 0.05). A similar correlation was noted at Mustoja ( $\rho = 0.99$ , p < 0.05). A strong negative correlation was evident between the C/N ratio and the sulphate concentration in lake water ( $\rho = -0.904$ ) at Kurtna.

## Diatom diversity and variability

Altogether 105 diatom species were identified, belonging to 36 genera. The average species richness per sample was  $17\pm0.7$ . Community diversity, indicated

by Shannon's H' value, was  $2.14\pm0.05$  and community evenness (Shannon's evenness) was  $0.76\pm0.01$ .

The overall species richness showed moderate variability between the study sites and areas (Tables 2 and 3). Rarefied species richness at Kurtna varied from 19 at the PK and K sites to 11 species at the J site, showing statistically significant differences between the study sites (F = 4.2, p = 0.002). Depending on the trophic status of the lake, statistically significant differences were found between species richness (F = 6.6, p = 0.003). The richness was significantly lower in the riparian zone of a soft-water eutrophic lake (site J) ( $11\pm0.8$ ) compared to dyseutrophic ( $17\pm0.8$ ) and hard-water mesotrophic ( $14\pm0.8$ ) lakes.

Shannon's diversity index was significantly higher at Mustoja ( $H = 2.45 \pm 0.1$ ) than at Kurtna ( $H = 2.03 \pm 0.1$ ) (t = -5.4, p < 0.001). The diversity index values also differed statistically (F = 4.2, p = 0.002) between the Kurtna sites; for example, study site J differed noticeably from the other lakesides having the lowest diatom diversity ( $H = 1.5 \pm 0.1$ ).

**Table 2.** Community characteristics of the Kurtna study sites (mean $\pm$ SE) with the trophic status of the water bodies and disturbance level. See Fig. 2 for the location of the study sites

Study site	Trophic status of the water body	Disturbance	Shannon's evenness	Shannon's diversity	Species richness
N1	MT	II	$0.77 \pm 0.04$	$2.15 \pm 0.1$	16±1.3
N2	MT	II	$0.78 \pm 0.05$	$2.15 \pm 0.2$	16±1.7
А	DT	II	$0.75 \pm 0.05$	$2.02 \pm 0.1$	$14\pm0.9$
S	DT	II	$0.72 \pm 0.02$	$1.92 \pm 0.04$	$14\pm0.8$
РК	DT	II	$0.78 \pm 0.05$	$2.33\!\pm\!0.2$	$19 \pm 1.9$
Κ	DT	II	$0.76 \pm 0.02$	$2.25\!\pm\!0.1$	$19\pm0.9$
J	ET	Ι	$0.62 \pm 0.05$	$1.49 \pm 1.3$	$11\pm0.8$
М	MT	Ι	$0.74 \pm 0.04$	$1.90\pm0.1$	$12\pm0.8$
Total			$0.74 \pm 0.02$	$2.03\!\pm\!0.1$	$15\pm0.6$

Abbreviations: DT – dyseutrophic; ET – soft-water eutrophic; MT – hardwater mesotrophic; I – Disturbance 1, alkaline air pollution; II – Distubance 2, alkaline air pollution and sulphate contamination.

Table 3. Community characteristics of the Mustoja study sites (mean  $\pm$  SE)

Study site	Shannon's evenness	Shannon's diversity	Species richness			
M1	$0.84 \pm 0.02$	$2.45 \pm 0.1$	$23 \pm 1.3$			
M2	$0.82 \pm 0.04$	$2.45 \pm 0.1$	$19 \pm 1.2$			
M3	$0.80 \pm 0.02$	$2.44 \pm 0.1$	$22 \pm 3.8$			
Total	$0.82\pm0.02$	$2.45\pm0.1$	$21 \pm 1.4$			

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The Mustoja sites (M1–M3) did not show statistically significant difference in diversity from one another (p = 0.9). At Kurtna the diatom diversity in the riparian zone of the soft-water eutrophic lake ( $H = 1.49\pm0.3$ ) was significantly (F = 9.1, p < 0.001) lower than near the hard-water mesotrophic ( $H = 2.07\pm0.1$ ) and dyseutrophic lakes ( $H = 2.13\pm0.1$ ).

Community evenness was slightly higher at Mustoja  $(0.82\pm0.02)$  than at Kurtna  $(0.74\pm0.2)$ , but the difference was not significant. While at Mustoja the evenness values showed little variability, site J at Kurtna with the lowest evenness  $(0.62\pm0.05)$  differed significantly (p < 0.05) from other Kurtna sites.

The riparian soil communities contained both aerial and freshwater algae. Both planktonic (e.g. *Aulacoseira granulata*) and periphytic (e.g. *Navicula cryptocephala*, *Fragilaria pinnata*) taxa were found from the Kurtna study area. At Mustoja planktonic species (e.g. *A. granulata* and *Stephanodiscus* spp.) were less common than periphytic taxa (e.g. *Eunotia exigua* and *Cocconeis* spp.). Common terrestrial species, present in both study areas, for example *Diadesmis contenta*, *Hantzschia amphioxys*, and *Pinnularia borealis*, were abundant and in many cases also the dominant taxa.

At Kurtna the most abundant genus was *Pinnularia* (relative abundance 0.16), followed by *Nitzschia* (0.14) and *Fragilaria* (0.12). Common species at Kurtna were *H. amphioxys* (0.14), *P. borealis* (0.11), *Stauroneis kriegeri* (0.08), and *Fragilaria nitzschioides* (0.06). At Mustoja the dominant genus was also *Pinnularia* (0.38). The most abundant species at Mustoja were *P. borealis* (0.19) and *P. lata* (0.14).

One of the most common species, *H. amphioxys*, was present at all study sites but not in every sample. The abundance of *H. amphioxys* varied between study sites but on average it measured approximately 22.3% of the diatom abundance in the samples. The percentage was the highest in study site A ( $55.3\pm24.1\%$ ) and the lowest in site N1 ( $9.9\pm4.4\%$ ).

*Rhopalodia gibba* was also present in all Kurtna sampling sites but constituted only a small percentage (on average 0.56%) of the diatom abundance. Together with *F. zeilleri* var. *elliptica R. gibba* was the most abundant in the N1 study site. They were also characteristic of the J site along with *Pinnularia perirrorata* and *Aulacoseira islandica*. In site A *D. contenta* was very abundant while *Pinnularia obscura* and *Diatoma tenuis* were common in site K. Otherwise less prevalent *P. lata* and *Achnanthidium kranzii* were characteristic of the M and J sites. The checklists of species (total abundance over 0.05%) showing their variability between study sites is presented in Table 4.

The species composition of most samples resembled that of the other samples collected from the same study site. For example, sites N1 and N2, which had similar diatom community properties, shared also several species (e.g. *R. gibba* and *F. zeilleri* var. *elliptica*). However, some samples had more shared taxa with other study sites than other samples from the same site, e.g. occasional samples from site K were more similar to samples from site M.

Despite the differences in the characteristics of water bodies between the study areas, the two areas had a partially common diatom community, which

**Table 4.** Occurrence of common taxa (overall abundance over 0.05%) in the study sites ('x' – present, '-' – absent). See Fig. 2 for the location of the study sites in the Kurtna Kame Field. M1–M3 – study sites in the Mustoja Kame Field

Species		Kurtna								Mustoja		
	J	М	Κ	PK	Α	S	N1	N2	M1	M2	M3	
Achnanthes conspicua		_	_			x	x	_	x	x	x	
Achnanthes exigua	_	_	_	_	_	_	_	_	x	x	x	
Achnanthes hungarica	x	_	x	_	_	x	_	_	_	_	_	
Achnanthes lanceolota	_	_	_	_	_	x	_	_	_	x	_	
Achnanthes minutissima		_	_	_	_	x	x	_	_	_	_	
Achnanthes nodosa		_	x	_	_	_	_	_	_	_	_	
Achnanthes trinodis	х	_	x	_	х	_	_	_	_	_	_	
Achnanthidium kranzii	х	х		х	х	х	х	х	х	_	_	
Aulacoseira crassipunctata	х	х	х	х	х	х	х	Х	_	_	_	
Aulacoseira granulata	х	_	х	х	х	х	_	х	х	_	х	
Aulacoseira islandica	х	_	_	х	_	_	_	_	_	_	_	
Caloneis molaris	_	_	х	_	_	х	х	_	_	_	_	
Cvclotella bodanica	_	_	х	_	_	_	х	х	х	х	х	
Čvclotella radiosa	х	_	х	х	х	х	_	_	х	х	х	
Ćvmbella affinis	х	х	х	х	_	х	х	х	_	_	_	
Ćymbella delicatula	_	х	_	_	_	_	х	_	_	_	_	
Diadesmis contenta	х	х	х	х	х	х	х	Х	х	_	х	
Diatoma tenuis	_	_	х	_	_	_	_	_	х	_	х	
Eucocconeis alpestris	_	х	_	_	_	_	_	_	_	_	_	
Eunotia binularis	х	х	х	х	х	х	х	х	х	х	х	
Eunotia exigua	х	х	х	х	_	х	х	х	х	х	х	
Eunotia incisa	х	_	х	_	_	х	_	_	х	_	х	
Eunotia paludosa	х	_	х	_	х	х	_	_	х	_	х	
Fragilaria capucina	х	х	х	_	х	х	Х	Х	х	х	х	
Fragilaria leptostauron	_	_	х	_	_	_	Х	Х	х	х	х	
Fragilaria nitzschioides	х	х	х	х	х	х	х	х	х	х	х	
Fragilaria pinnata	х	-	х	—	х	-	Х	Х	_	_	—	
Fragilaria zeilleri var. elliptica	х	х	х	х	х	-	Х	Х	х	х	х	
Fragilariaforma bicapitata	_	_	х	_	_	-	-	-	х	-	Х	
Fragilariaforma virescens	х	х	х	х	Х	Х	х	х	х	х	Х	
Geissleria ignota	-	х	-	х	-	-	-	Х	-	-	-	
Hantzschia amphioxys		х	х	х	Х	Х	х	х	х	х	Х	
Navicula absoluta	х	х	-	-	Х	-	Х	-	-	-	-	
Navicula cari		-	-	_	-	-	-	х	Х	Х	-	
Navicula constans var. symmetrica		х	-	-	-	-	-	-	_	_	-	
Navicula cryptocephala	-	х	-	_	Х	Х	х	х	-	-	-	
Navicula mutica	-	х	х	-	х	-	-	Х	Х	х	Х	
Navicula oblonga	-	-	х	х	-	-	_	-	-	-	-	
Nitzschia amphibia	-	-	х	х	х	х	Х	Х	-	-	-	
Pinnularia borealis	х	х	х	х	х	х	Х	Х	Х	х	Х	
Pinnularia divergentissima		х	х	х	Х	х	х	х	Х	х	Х	
Pinnularia lata		х	х	_	-	-	-	-	Х	х	Х	
Pinnularia obscura		х	х	х	Х	Х	х	х	Х	х	Х	
Pinnularia perirrorata		х	х	х	Х	х	х	х	х	Х	Х	
Khopalodia gibba		-	х	х	-	Х	х	_	х	х	Х	
Stauroneis kriegeri		х	х	х	Х	х	х	х	х	Х	Х	
Stauroneis amphicephala		х	х	х	Х	х	Х	х	Х	Х	х	
Staurosirella leptostauron		-	-	-	-	-	Х	Х	_	-	-	
Synedra ulna		X	X	х	х	х	X	X	Х	Х	х	
Tabellaria fenestrata	_	X	X	-	-	-	Х	X	_	-	-	
Tabellaria flocculosa	_	X	X	-	-	-	_	X	_	X	Х	

included about 51.4% of the species. In addition to the widespread *P. borealis* and *H. amphioxys*, also *Fragilariaforma virescens*, *P. perirrorata*, and *Stauroneis amphicephala* were among these shared species.

#### Community relation to environmental parameters

The forward selection of variables into CCA gave six significant (p < 0.05) variables explaining the variation in diatom community: N content (p < 0.001), C/N ratio (p = 0.02), Mg content (p = 0.004), disturbance level (p = 0.006), carbonate content (p = 0.012), P content (p = 0.012) (Fig. 3). These six explanatory variables



**Fig. 3.** CCA ordination diagram of the Kurtna diatom communities. Environmental factors explain 28% of the variation in species data. Abbreviations of environmental factors: *C/N* – carbon nitrogen ratio, *Carb* – carbonate content, *Mg* – magnesium content, *P* – phosphorus content, *Disturbance 1* – air pollution, *Disturbance 2* – air pollution and water contamination. Abbreviations of species names: AE – Achnanthes exigua, AH – A. hungarica, ACNO – A. nodosa, ACHK – Achnanthidium kranzii, AUG – Aulacoseira granulata, AUI – A. islandica, CAM – Caloneis molaris, CB – Cyclotella bodanica, CR – C. radiosa, CYM – Cymbella spp., CYMA – C. affins, CYMD – C. delicatula, DC – Diadesmis contenta, DT – Diatoma tenuis, EU – Eunotia spp., EUB – E. binularis, EUEX – E. exigua, EUP – E. paludosa, FC – Fragilaria capucina, FL – F. leptostauron, FP – F. pinnata, FZE – F. zeilleri var. elliptica, FFV – Fragilariaforma virescens, HA – Hantzschia amphioxys, NAAB – Navicula absoluta, NACR – N. cryptocephala, NM – N. mutica, NAOB – N. oblonga, NIAM – Nitzschia amphibia, PB – Pinnularia borealis, PD – P. divergentissima, PL – P. lata, PO – P. obscura, PP – P. perirrorata, RHOG – Rhopalodia gibba, STLEP – Staurosirella leptostauron, STAM – Stauroneis amphicephala, TF – Tabellaria fenestrata, TFL – T. flocculosa.

explained 28% of the variation in species data with the first two ordination axes explaining 11% and 6% of the variation, respectively.

Species that are associated with the less disturbed sites (Disturbance 1) are e.g. *A. kranzii, Tabellaria fenestrata,* and *T. flocculosa.* Species that are associated with the second disturbance level (Disturbance 2) are e.g. *Achnanthes exigua, F. pinnata,* and *Nitzschia amphibia.* Species related to soil C/N ratio are *E. exigua, Navicula mutica, P. borealis,* and *P. perirrorata.* Numerous species also correspond with the levels of P, N, and Mg, e.g. *A. granulata* appears to be related to P and *Eunotia paludosa* to N content.

Not all environmental parameters were included in the CCA. Additionally, the variability of some most abundant taxa was explored against the remaining environmental parameters. The abundance of *F. zeilleri* var. *elliptica* and *R. gibba* was significantly positively correlated with the lake sulphate concentration ( $\rho = 0.72$ , p = 0.03;  $\rho = 0.73$ , p < 0.001, respectively) while *P. lata* was present only when the sulphate concentrations were low.

# Diatoms as potential indicators of anthropogenic disturbance: levels and gradients in the Kurtna study area

Comparison of the two anthropogenic disturbance levels of the Kurtna study sites revealed some differences in the diatom community composition. At the higher disturbance level (Disturbance 2), the abundance of *H. amphioxys*, *D. contenta*, and *N. amphibia* was higher. These sites also had the lowest abundance of *F. virescens* and *P. lata* as well as of the genera *Cymbella*, *Lemnicola*, and *Tabellaria*. Most of the other species, among them *P. borealis*, were more abundant at the lower disturbance level (Disturbance 1).

The species richness of the sites influenced solely by alkaline air pollution was 11 but on sites influenced by both factors, the species richness was higher at 16. The diversity at the two levels was similar ( $H=1.70\pm0.3$  at Disturbance 1 and  $2.13\pm0.3$  at Disturbance 2). Evenness was higher at the level of multiple disturbances compared to the lower level ( $0.76\pm0.02$  and  $0.68\pm0.04$ , respectively). The differences were statistically significant in all cases (diversity t=-3.8, p < 0.001; species richness t=-4.4, p < 0.001; evenness t=-2.4, p=0.02). ANOVA revealed a significant difference in the values of Shannon's diversity index between different water contamination (SO<sub>4</sub>) levels (F=5.2, p=0.001). Also the species richness differed significantly (F=5.6, p=0.001) between sulphate concentration levels. Evenness did not show any significant differences between sulphate concentration levels (p=0.067).

## DISCUSSION

Considering the overall differences and similarities of the sampling sites, the number of species and the diversity encountered in this study were relatively high compared to previous studies of soils in Estonia (Vacht, 2012). Based on this

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study, we can conclude that the riparian soils have a high abundance of diatom flora. This is most likely due to sufficient moisture availability caused by the influence of the water body on the riparian zone. Moisture is otherwise often considered a limiting factor to diatoms (Camburn, 1982; Ito and Horiuchi, 1991; Van de Vijver et al., 2002). The various habitat preferences of the taxa found illustrate the presence of miscellaneous microhabitats in the riparian soils.

In previous studies of the two study areas an evident increase in the Kurtna topsoil pH was noted (Kont et al., 1994). Differences in riparian topsoil pH values between Kurtna and Mustoja were present, but not significant. A possible reason is the decrease in alkaline air pollution in recent years and/or the location of the study sites on rather low landforms at Kurtna that could have received less of the fly-ash deposition. Even though the differences in the pH were less significant than expected, pH is still likely to influence other soil parameters such as base saturation rate or hydrolytic acidity (Kont et al., 1994) and through these the habitat properties.

The trophic status of the soil, reflected by the C/N ratio (Kont et al., 1994), was high at both Kurtna and Mustoja. Generally Kurtna study sites had a higher nutrient content than the Mustoja sites. This implied better growing conditions for higher plants at Kurtna, and might even suggest better nutrient availability for soil algae. This, however, does not solely reflect the conditions of the habitat as the possible effects of trace substances from the alkaline deposition should also be taken into consideration.

Neither the elevated carbonate content in the riparian soils nor the occasionally low organic matter content seemed to affect the diatom community parameters at Kurtna. For example, the diatom diversity was close to the overall mean value even at the PK site, where the carbonate content was the highest. The sites with the highest organic matter content however had the highest diversity and species richness. Despite the moderate levels of organic matter, all of the community parameters (diversity, richness, and evenness) were still higher at Mustoja. The exceptionally low community characteristics at site J at Kurtna can not be explained solely by the measured soil parameters, but could be caused by the trophic level of the lake, which limits the diversity, evenness, and species richness of the riparian diatom community.

The species found abundantly in the riparian soils, e.g. *D. contenta*, *H. amphioxys*, and *P. borealis*, are known to live in extreme environments and to survive even those demonstrating desiccation and freezing (Souffreau et al., 2010). Their abundance in the environmentally variable riparian zone was therefore not surprising. *Achnanthes minutissima* has also been found in aquatic habitats subject to extensive amounts of pollution (Kobayasi and Mayama, 1982), other species such as *E. paludosa* and *T. flocculosa* have been frequently found in ombrotrophic mires (Poulíčková et al., 2013) or together with *N. cryptocephala*, *Gomphonema parvulum*, and *N. amphibia* in wetlands under intensive human influence (Weilhoefer and Pan, 2007). Most of these species were present in both study areas, and cannot be considered to reflect the anthropogenic disturbances but are related to the fact that they are tolerant species found in various soil

ecosystems. However, the absence of *A. minutissima*, *N. cryptocephala*, and *N. amphibia* at Mustoja may suggest that these species are related to the higher overall disturbance level at Kurtna. Alterntively, they could be related to the differences in water chemistry. Species characterizing the Mustoja study area were the fairly unresearched *A. kranzii* and epipelic *P. lata*, which should be studied in more detail before their possible indication abilities in this context can be evaluated.

The high dominance of e.g. *A. minutissima* with wide tolerance limits in this study could be a sign of difficult habitat conditions influenced by multiple disturbance factors. In contrast, the richness of taxa showed that the habitats have fairly high biodiversity. The high percentage of shared species (among them e.g. *P. perirrorata*) in both study areas may reflect the similarities between these areas, i.e. development, vegetation characteristics, moisture conditions, presence of microhabitats, etc. Whether the rest of the species present in the Mustoja study area are present due to for example lack of anthropogenic disturbances or differences in water chemistry, remains to be studied.

In most cases, the species composition in single samples resembled that of the other samples collected from the same study site. However, in some cases the similarities were more notable compared to other study sites. This indicates a high variability in diatom species composition even within a single riparian area.

Diatom communities varied under different levels of anthropogenic disturbances. The general diatom community parameters however were not always influenced in the expected manner; e.g. diatom species richness was higher at the higher disturbance level (Disturbance 2). Species richness varied to some extent along the mining water gradient, reaching the highest values at intermediate levels. A large number of species were indifferent to the disturbance level and even to the sulphate concentration gradient. We can, therefore, suggest that even though the diatom communities as a whole react to various disturbances, the effect is more evident on species level, e.g. P. lata was present when the disturbance level was low. The strong correlation between e.g. R. gibba and sulphate concentrations could be influenced by the fact that the R. gibba was highly abundant on the N1 study site, which had one of the highest sulphate concentrations and could, therefore, be an artefact from our sampling design. Alternatively, its abundance could be linked to high carbonate or low organic matter content. As the species was also present, though not abundantly, at Mustoja, it is doubtful that the species has a strong indicative value for sulphate concentrations.

Knowing that riparian areas tend to have high biodiversity, the variability and abundance of the diatom flora in the studied soils was not surprising. The changing moisture conditions and variable microhabitats enable the variability of diatom taxa in riparian soils to range from aquatic to terrestrial species. Community properties such as diversity and species richness are promising factors for indicative purposes especially in the case of long-term disturbances. Therefore the finding that the diversity, evenness, and species richness were hardly affected by human influence was unexpected. Depending on the study area and site, and through them, anthropogenic disturbance levels, and gradients, the species composition varied. This indicates that the answer to monitoring this type of human influence probably lies in species specific indicator properties rather than community properties. In this case, however, the variability of diatom community parameters and structure between these study areas and sites may be too great to propose certain bioindicators without further studies.

## CONCLUSIONS

Diatom diversity, evenness, species richness, and community composition varied moderately between the study sites and study areas. The variability of riparian diatom communities was most likely influenced by the water and soil parameters and even through the variable levels of anthropogenic disturbance and gradients. Diatom community diversity, evenness, and species richness increased with increasing disturbance level; on the sulphate contamination gradient the trend was not so evident. Possible indicators of anthropogenic disturbances could be the dominance of *Hantzschia amphioxys*, a high abundance of *Fragilaria zeilleri* var. *elliptica* and *Rhopalodia gibba*, and a low abundance of *Pinnularia lata* in case of disturbances rather than general community properties. The two study areas had a common core community (composing over 50% of the species present), which indicates similarities of the two areas, at the same time suggesting that the remainder of the communities could possibly indicate the differences, anthropogenic or natural, between the areas.

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