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Iron forms and peroxidase activity in forest island soils

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Abstract. Oxidation–reduction reactions play a key role in ecologically important biogeochemical processes in soil and influence soil chemical, biochemical, and biological properties. The objective of this study was to compare the effect of a forest island located on two kinds of soils on the concentrations of iron forms and peroxidase activity participating in oxidation–reduction processes. The investigations were carried out in the Agroecological Landscape Park in Turew (40 km south of Poznań, Western Polish Lowland, 16°45′E, 52°01′N). The subject of the study was a palace park, which can be considered as a forest island. Part of this forest island is located on mineral soil and another part on mineral–organic soil. Our results showed that the flow of groundwater was accompanied by an increase in peroxidase activity, total iron concentration, and Fe(II) and Fe(III) ions in the mineral soils in most periods of sampling. However, an increase of peroxidase activity and a decrease of total iron concentration and Fe(II) and Fe(III) ions accompanying the flow of groundwater were observed in the mineral–organic soils.

Key words: forest island, ferrous ions, ferric ions, peroxidase activity.

INTRODUCTION

Forest islands as biogeochemical barriers are a very important element of the landscape structure. In agricultural landscape large amounts of migrating nutrients are leached out from cultivated soils. As the well-developed root system of trees contains more groundwater in its effective range than the root system of cereal plants, trees have a strong limiting effect on the spreading of chemical pollution in groundwater. In addition, forest islands belong to permanent elements in landscape, which restrain erosion in soil, separate agricultural fields from the watercourses, improve microclimate for agricultural production, and help in maintaining biodiversity of agricultural fields (Ryszkowski & Bartoszewicz, 1989; Ryszkowski et al., 1999).

Chemical, biochemical, physical, and biological processes in soil organic matter have a catalytic character. Thus these pathways and their mechanisms occurring in soil organic matter are significantly dependent on the properties of the environment.

Oxidation–reduction reactions play a key role in ecologically important biogeochemical processes in soil and influence soil chemical, biochemical, and biological properties. The redox potential is a critical environmental factor because it governs the chemical and biochemical form of many compounds and their availability for plants and soil microorganisms, and it also influences the products of microbial metabolism in soil. Furthermore, oxidation and reduction of organic matter are intimately linked with energy transformations, which may form the basis of an energy-yielding metabolism (Harrison, 1992; Gliński et al., 1996; Tan, 2003).

Organic matter is capable of inducing reduction and oxidation reactions, hence affecting the redox system in the environment. Therefore, humic substances are important components of the soil redox systems, capable of transferring electrons. The presence of organic matter has great influence on the bioavailability and mobility of iron in forest soils. Polyvalent metals such as iron can form very stable complexes by binding to multiple functional groups on one dissolved organic matter molecule and are perhaps the most versatile of the biocatalytic elements (Peretyazhko & Sposito, 2006).

Furthermore, iron is bound to sulphurs in polypeptides: bisulphide, sulphhydral, and sulphate groups. Iron–sulphur clusters are prosthetic groups commonly found in various proteins. They participate in oxidation–reduction pathways and catalysis. The proteins belonging to this group are ferredoxins, which represent the iron–sulphur cluster that performs the intermediate step in the electron transfer chain, transferring electrons from the flavoprotein to the terminal dioxygenase (Bertini et al., 1996). Complexation of iron in forest soils is influenced by several soil solution variables, including the redox potential and the solution pH value. However, the redox potential determines the Fe(II)/Fe(III) speciation (Straub et al., 2001; Pedersen et al., 2005). It is likely that micronutrients are predominantly transported to plant roots as soluble chelate complexes. In particular, Fe deficiencies are more widespread than those of Mn, Zn, and Cu (Lindsay & Schwab, 1982). According to Moghimi et al. (1978) and Chen (1996), soluble organic complexes of iron play an important role in supplying micronutrients to plants. These soluble organic compounds consist of root exudates, humic substances, metabolites of microorganisms, or applied iron–chelate fertilizers.

The spectrophotometric determination of Fe(II) and Fe(III) is usually preceded by their separation from major components and from interfering elements, the effects of which cannot be eliminated by other methods such as masking or changing the pH of the medium. Spectrophotometry enables one to determine, with good precision and sensitivity, almost all the elements present in small and trace quantities in soil. Amongst many spectrophotometric methods for the measurements of iron in soil, thiocyanate, 1,10-phenanthroline, and bathophenanthroline are generally used (Marczenko et al., 2000). According to Marczenko et al. (2000), Fe(III) thiocyanate complexes are not very stable and can persist only at a relatively high concentration of SCN^-. The dye of aqueous solutions of Fe(III) thiocyanate complexes is unstable owing to the reduction of Fe(III) by SCN^-, fading by a few per cent in 30 min and by 50% in 6 h. However, in the phenanthroline method solutions of the complexes with *o*-phenanthroline are stable, and the Fe(III) bound

in the complex is resistant to oxidation. The complex of 1,10-phenanthroline with Fe(II) has been widely used in titrimetric analysis as a redox indicator and also for determining higher concentrations of iron. Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) reacts with Fe(II) ions very similarly to 1,10-phenanthroline. Both methods are of similar selectivity; however, the bathophenanthroline method is much more sensitive. Moreover, Goswami & Kalita (1988) observed that in the bathophenanthroline method there is scope for losses during solvent extraction steps, whereas in the 1,10-phenanthroline method there is scope for losses during transfer to the volumetric flask, and the method requires several steps including accurate adjustment of pH. Tarafder & Thakur (2005) suggest the use of the thiocyanate and 1,10-phenanthroline method for the determination of Fe ions in soil, silicate rock, minerals, stream sediments, and water samples from sub-microgram to percentage level. The method is characterized by high sensitivity, selectivity, reproducibility of results, and accuracy.

However, the most sensitive technique for the determination of sub-nanomolar levels of Fe(II) is flow injection analysis with chemiluminescence detection. Since there was no separation of Fe(II) and Fe(III) in the analyses, the measurements of Fe(II) concentration have to be corrected for the chemi-luminescence by Fe(III). Most fluorescent methods for the determination of Fe(II) exhibit low sensitivity due to fluorescence quenching upon binding of Fe(II) (Chen et al., 2006). In addition, ion chromatography for the analysis of ferrous and ferric ions in soil extracts was developed by Ye et al. (1998). Both ferric and ferrous ions can be analysed directly and simultaneously. This study revealed no interference in high pressure liquid chromatography analysis from the common cations calcium(II), magnesium(II), and aluminium(III).

Soil organic matter transformation is strongly affected by the activities of soil microorganisms, which use many enzymes in their metabolic pathways. Peroxidases are present in natural soil and may originate from microorganisms, plants, or other organisms. This enzyme has been the most studied because of its role in organic matter degradation and release of nutrients in soil. Peroxidase is apparently involved in the humification processes. The oxidative matalloenzyme is able to oxidize aromatic substrates with a high redox potential and has unique catalytic properties. Peroxidase catalyses the oxidation of phenols and aromatic amines in the presence of hydrogen peroxide as an electron acceptor in the reactions. The release of carboxyl and methoxyl groups from phenolic substrates is ascribed mainly to microbial activity and may lead to CO_2 production in soil (Bollag et al., 1987; Nicell & Wright, 1997; Choinowski et al., 1999; Criquet et al., 2000; Dec et al., 2003).

Peroxidase activity in soil was first reported by Galstyan (1958). According to the Galstyan procedure, soil was extracted by toluene and the filtrate was incubated with pyrogallol and H_2O_2 at 28 °C. The reaction was stopped by the addition of 20% H_2SO_4 and the purpurogallin was extracted by ethyl ether. In spite of repeated and painstaking attempts, it was not possible to detect significant peroxidase activity in experimental soils using the technique described by Galstyan (1958). Purified horseradish peroxidase, when subjected to the same treatment

that he used for the extraction of his soil samples, lost 91% of its original activity. This resulted in high and erratic blank readings, which bracketed the reading of the active samples. However, Kozlov (1964) used pyrocatechol instead of pyrogallol for peroxidase assay. Moreover, peroxidase activity in soil assay by the Bartha & Bordeleau (1969) method proved to be quick, sensitive, and highly reproducible. According to these authors, the quick and accurate assay should prove helpful in research and is being used with success for establishing correlations between peroxidase activity with soil type, microbial counts, and pesticides (Bartha et al., 1968; Bordeleau & Bartha, 1972).

Our previous studies dealt with the problem of the influence of a forest island in agricultural landscape on the changes of total nitrogen content, average yearly concentration of ammonia, nitrate ions, activity of urease, as well as the chemical structure of humic acids (HA) in order to understand their role in the functioning of forest islands as biogeochemical barriers. However, the studied forest island is located on two kinds of soils: mineral and organic (Szajdak et al., 2002a, 2002b; Meysner et al., 2006). In those studies the chemical structure of HA was investigated using several analytic techniques: EPR, UV-VIS, ^{13}C NMR, and thermal. In mineral soil, a decrease was observed in the intensity of the organic signal in EPR spectra of the HA, indicating a decrease in the dimensions of the aromatic polyconjugation systems in their molecules accompanying the flow of groundwater. These data are in good accordance with the results of UV-VIS spectroscopy. The higher optical densities at E_4/E_6 ratios for the HA from mineral–organic soils compared to those from mineral soils confirm a higher degree of condensation and polyconjugation in the molecules of the former. In mineral soils, an increase accompanying the flow of groundwater leads to a rise of carbohydrate C and C in the carbonyl-containing group, predominantly carboxylic in the HA. In mineral–organic soils, a reduction of C in little modified fragments of lignin and a significant rise of carbohydrate C in the humic molecules accompanied the flow of groundwater. Thermal properties of the HA in both soils indicated an increase in the content of thermolable structural units (carbohydrates, free and bound functional groups) and a decrease in the thermostable skeleton part of the HA molecules with an increase in the direction of the flow of groundwater. For both kinds of soils, an increase following the flow of groundwater was accompanied by a decrease in the degree of humification or chemical maturity of HA.

The present work is a continuation of our earlier studies. The objective of this study was to compare the effect of the forest island located on two kinds of soils on the concentrations of iron forms and peroxidase activity participating in oxidation–reduction processes.

MATERIALS AND METHODS

The investigations were carried out in the Agroecological Landscape Park in Turew (40 km south of Poznań, Western Polish Lowland, 16°45′E and 52°01′N). The subject of the study was a palace park that can be considered as a forest

island. Soil samples were taken from four sites, marked as 1, 2, 3, and 4, selected near wells. Site 1 is located on the border between a cultivated field and the forest island. The distances between the sites were as follows: site number 1 is situated 57.4 m from site 2, site 2 is located 47.0 m from site 3, and the distance between sites 3 and 4 is 10.5 m (Fig. 1). Part of this forest island is located on mineral soil and another part on mineral–organic soil. Table 1 gives the classification of soils according to the Polish Systematic. The soils were characterized by grain-size distribution; the top layers are formed of sand with varying content of fine particles while in deeper layers (from 60 to 80 cm) sandy loam and silty loam predominate. At sites 3 and 4 mineral–organic soils belonging to mucky soils appear. The following trees are growing on the investigated transect of the forest island: *Quercus robur*, *Fraxinus excelsior*, *Acer platanoides*, *Robinia pseudoacacia*, *Ulmus laevis*, *Fagus sylvatica*, *Tilia platyphyllos* (Table 2).

Samples were taken from the layer of 0–20 cm after removal of leaf litter from March to November 2008. Four soil samples from each site were pooled together to obtain an average mixed sample. Roots and stones were removed. Samples were air-dried and crushed to pass through a 1 mm mesh sieve. Total organic carbon (TOC), dissolved organic carbon (DOC), ferrous (Fe(II)) and ferric (Fe(III)) ions,

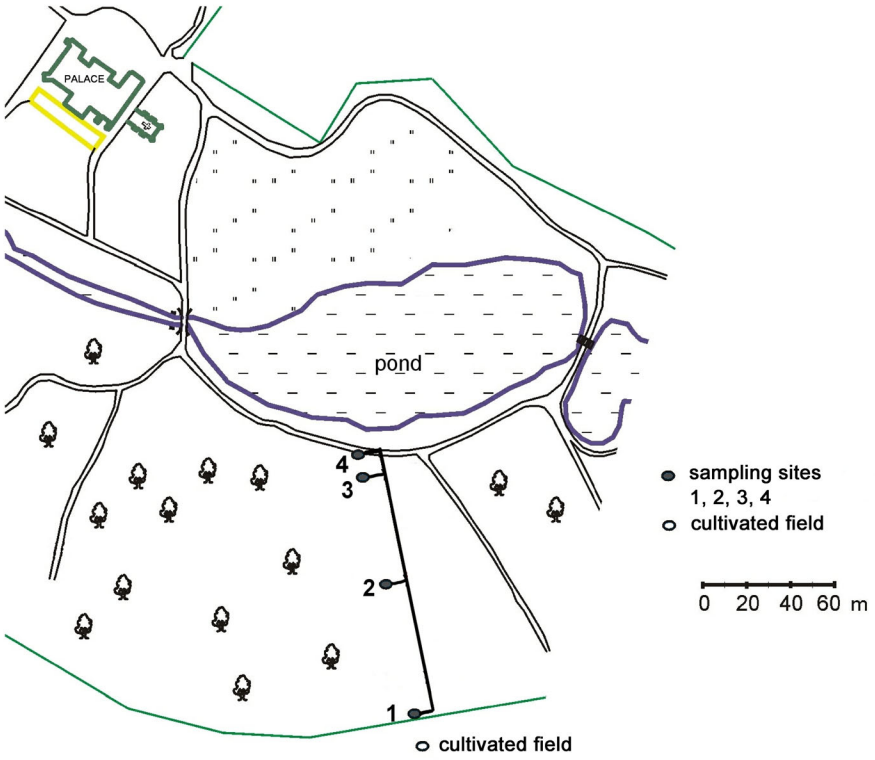

Fig. 1. Agroecological Landscape Park in Turew.

Table 1. Classification and some physical and chemical properties of mineral (1, 2) and mineral–organic (3, 4) soils in 2008

Sampling site	Classification of soils	Percentage of mechanical fractions (diameters in mm)						Soil texture	Groundwater level, cm	Total N, g kg^{-1}	C/N
		2–0.1	0.1–0.005	0.005–0.02	0.02–0.005	0.005–0.002	<0.002				
1	D – autogenic soil O – brown forest soil T – grey-brown podzolic soil S – glossic soil	71	7	5	3	3	11	Heavy loamy sand	110–220	1.41–2.39	9–14
2	D – autogenic soil O – brown forest soil T – grey-brown podzolic soil S – gleyed soil	74	7	3	3	3	9	Light loamy sand	110–201	0.63–1.48	8–18
3	D – hydrogenic soil O – post-bog soil T – mucky soil S – muckous soil	61	11	8	3	5	12	Heavy loamy sand	120–225	1.65–2.71	9–17
4	D – hydrogenic soil O – post-bog soil T – mucky soil S – muckous soil	60	9	10	3	5	13	Light loam	125–185	1.98–4.56	6–14

D – division, O – order, T – type, S – subtype.

Table 2. Species of plants on mineral (1, 2) and mineral–organic (3, 4) soils in 2008

Sampling site	Forest vegetation layers	Species of plants
1	Canopy	*Quercus robur*, *Fraxinus excelsior*, *Acer platanoides*, *Robinia pseudoacacia*, *Ulmus laevis*
	Understory	*Sambucus nigra*, *Crataegus monogyna*
	Forest floor	*Alliaria petiolata*, *Stachys sylvatica*, *Quercus robur*, *Viola odorata*, *Chelidonium majus*, *Fraxinus excelsior*
2	Canopy	*Fagus sylvatica*
	Understory	*Sambucus nigra*, *Quercus robur*, *Carpinus betulus*, *Fraxinus excelsior*, *Acer platanoides*
	Forest floor	*Hedera helix*, *Quercus robur*, *Fraxinus excelsior*, *Acer platanoides*
3	Canopy	*Tilia platyphyllos*
	Understory	*Sambucus nigra*, *Acer platanoides*
	Forest floor	*Hedera helix*, *Geranium robertianum*, *Stachys sylvatica*, *Geum urbanum*, *Adoxa moschatellina*, *Aegopodium podagraria*
4	Canopy	*Fraxinus excelsior*, *Ulmus laevis*
	Understory	*Sambucus nigra*, *Crataegus monogyna*, *Acer platanoides*
	Forest floor	*Hedera helix*, *Stachys sylvatica*, *Viola odorata*, *Ranunculus lanuginosus*, *Acer platanoides*, *Geum urbanum*, *Aegopodium podagraria*, *Galium aparine*, *Chaerophyllum temulum*, *Chaerophyllum aromaticum*

and pH were determined. Total Fe is the sum of Fe(II) and Fe(III) ions. Soil pH was measured in soil 1 M KCl (1 : 5 v/v) suspensions applying the potentiometric method. TOC was analysed on Total Organic Carbon Analyzer (TOC 5050A) with Solid Sample Module (SSM-5000A) produced by Shimadzu (Japan). For the investigation of hot water extractable carbon the soil samples were heated in bidistilled water at 100°C for 2 h under a reflux condenser. Extracts were separated by the medium porosity filter paper and analysed on TOC 5050A facilities. Bidistilled water from silica glass equipment was used (Smolander & Kitunen, 2002). For analyses of peroxidase activity fresh soil from each site was pooled together to obtain an average mixed sample. The tested soil was screened through a sieve with 2.0 mm mesh.

The further procedure of the investigations followed the term 'the direction of the flow of groundwater', which indicated conventionally changes in the concentrations of the examined compounds along the direction of the groundwater flow.

Peroxidase activity in soils was determined by the Bartha and Bordeleau method (Bartha & Bordeleau, 1969). Horseradish peroxidase Type II (Sigma) was used as enzyme standard. Horseradish peroxidase (0.1 g of standard) was dissolved in 0.05 M phosphate buffer at pH = 6 in a 1000 mL volumetric flask to prepare standard stock solution. The calibration curve was constructed by adding 1.0, 2.0,

3.0, 4.0, and 5.0 mL of working standard in five 25 mL volumetric flasks. Then 3.0 mL of each standard, 0.5 mL of 0.06% H_2O_2 in 0.05 M phosphate buffer at pH = 6, and 0.1 mL of 0.5% *o*-dianisidine in methanol were combined in a 1 cm spectrophotometric cuvette for colorimetric analysis. All calibration standards were mixed and allowed to stand for 10 min at 20 °C. The absorbance of the solution was measured colorimetrically at $\lambda = 460$ nm using a Beckman DU®-68 (USA) UV-VIS spectrophotometer. Peroxidase activity in soils was calculated from the earlier prepared analytical curve according to the Lambert–Beer light absorption law by means of the least squares formula (1) (Fig. 2, Table 3).

$$A = \varepsilon c\, l, \tag{1}$$

where A – absorbance; ε – molar absorption coefficient, L mol^{-1} cm^{-1}; c – concentration, mol L^{-1}, and l – thickness of layer (1 cm).

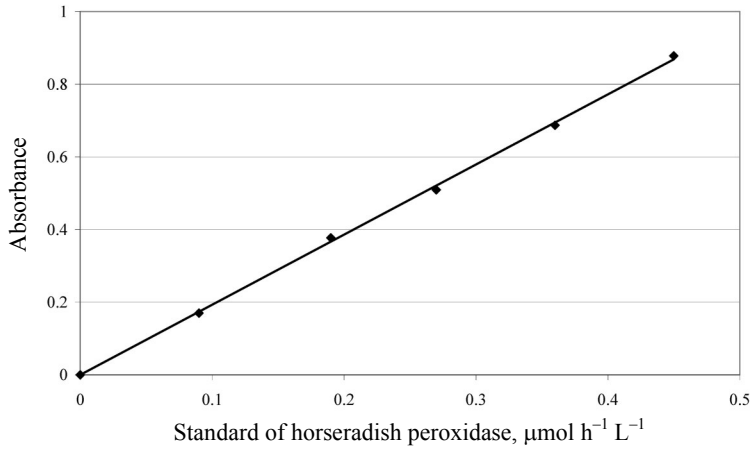

Fig. 2. Analytical curve of the concentrations of horseradish peroxidase.

Table 3. Parameters of Lambert–Beer calibration curve according to formula (1)

Index	Molar absorption coefficient (ε), L mol^{-1} cm^{-1}	Correlation coefficient (r)
Peroxidase activity	191 799 ± 3	0.997
Iron(II)	5 500 ± 3	0.999
Iron(III)	6 277 ± 4	0.999

$\bar{x} \pm \Delta X$ – confidence interval of average at confidence level $\alpha = 0.05$ for $n - 1$ degrees of freedom.

According to the procedure, 20 g of soil was suspended in 100 mL of 0.05 M phosphate buffer at pH = 6 in a flask closed with a stopper, shaked on a rotary shaker, and incubated for 1 h at 25 °C. Thereafter, the samples were centrifuged at 4000 rpm for 20 min, and the soil solutions were filtered. The peroxidase assay was as follows: 0.5 mL of 0.06% H_2O_2 in 0.05 M phosphate buffer at pH = 6, 0.1 mL of 0.5% *o*-dianisidine in methanol, and 3 mL of soil extract were combined in a 1 cm spectrophotometric cuvette. All the samples were kept at 20 °C for 10 min. The ingredients were mixed and the increase in the absorbance was continuously recorded at $\lambda = 460$ nm. Heat inactivation achieved by placing the flasks containing the soil extract into a 100 °C water bath for 5 min served as control. Further the procedure was performed as in the case of the tested sample but without H_2O_2.

The ferrous ions in soils were determined by the phenanthroline method (Minczewski & Marczenko, 1976). In order to prepare standard stock solution 0.0485 g of $FeCl_3 \cdot 6H_2O$ was dissolved in bidistilled water and the volume was diluted to 100 mL with bidistilled water in a volumetric flask in which 1.0 mL of this solution contained 0.1 mg of Fe(III). The calibration standards were constructed by adding 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL of working standard in six 50 mL volumetric flasks. To each standard 2.0 mL of 10% $NH_2OH \cdot HCl$, 5.0 mL of 10% $C_6H_5Na_3O_7$, and 5 mL of 0.25% *o*-phenanthroline were added for colorimetric analysis. All calibration solutions were mixed and allowed to stand at 20 °C for 10 min. The concentrations of ferrous ions were determined colorimetrically at $\lambda_{max} = 512$ nm on a Shimadzu UV-VIS spectrophotometer UV-mini 1240. Bidistilled water was used as the blank.

The concentration of Fe(II) was calculated from the previously prepared analytical curve using standard solutions of Fe(II) according to the Lambert–Beer light absorption law by means of the least squares formula (1) (Fig. 3, Table 3).

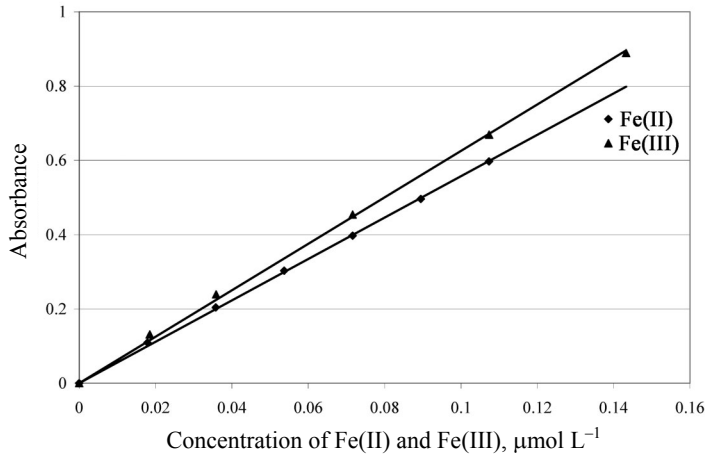

Fig. 3. Analytical curve of the concentrations of ferrous and ferric ions.

According to the procedure, 50 g of soil was extracted with 50 mL of bidistilled water and placed in a flask closed with a cork and agitated on a rotary shaker for 1 h. Samples were centrifuged at 4000 rpm for 20 min, and soil suspensions were filtered. Next the concentration of Fe(II) in soils was assayed as follows: 5 mL of 0.25% *o*-phenanthroline and 15 mL of soil extract were combined in 25 mL volumetric flasks. Then the flask was topped up with bidistilled water, and the contents were thoroughly stirred. The samples were stored at 20°C for 10 min. The concentrations of ferrous ions were calculated from the change of the absorbance per minute at λ_{max} = 512 nm with relation to the molar absorption coefficient of Fe(II).

The ferric ions were estimated by the thiocyanate technique (Minczewski & Marczenko, 1976). In order to prepare standard stock solution 0.0485 g of $FeCl_3 \cdot 6H_2O$ was dissolved in bidistilled water and the volume was diluted to 100 mL with bidistilled water in a volumetric flask where 1.0 mL of the solution contained 0.1 mg of Fe(III). The calibration standards were constructed by adding 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mL of the standard solution into 50 mL volumetric flasks. Next, 0.5 mL of 2M HNO_3 and 3.0 mL of 10% KSCN were added to each flask and the flasks were filled up to a mark with bidistilled water. All calibration solutions were mixed and allowed to stand at 20°C for 10 min. The concentration of ferric ions was determined colorimetrically at λ_{max} = 480 nm on a Shimadzu UV-VIS spectrophotometer UV-mini 1240. Bidistilled water was used as the blank.

The concentration of Fe(III) was calculated from the previously prepared calibration curve using calibration standards of Fe(III) according to the Lambert–Beer light absorption law by means of the least squares formula (1) (Fig. 3, Table 3).

According to the procedure, 50 g of soil was extracted with 50 mL of bidistilled water, placed in a flask closed with a cork, and agitated on a rotary shaker for 1 h. Samples were centrifuged at 4000 rpm for 20 min, and soil suspensions were filtered. Then 15 mL of soil extract, 0.5 mL of 2 M HNO_3, and 3.0 mL of 10% KSCN were combined in a 25 mL measuring flask. After that the volume was topped with bidistilled water for colorimetric analysis. The samples were allowed to stay at 20°C for 10 min. Then the absorbance of the reaction mixture was measured at λ_{max} = 480 nm.

All the experiments were run in triplicate, and the results were averaged. The confidence intervals were calculated using the following formula: $\bar{x} \pm t_{\alpha(n-1)}\mathrm{SD}$, where $\bar{x}$ – mean, $t_{\alpha(n-1)}$ – value of the Student test for $\alpha = 0.05$, and $n-1$ degree of freedom, SD – standard deviation. Linear correlations between the values were calculated. All the chemicals used in this study were of analytical grade of purity.

RESULTS AND DISCUSSION

The most important physicochemical parameter of the soil is pH, which affects plant growth and the behaviour of the contaminants in soils: the present study showed considerable differences in the pH values of the two soils; the pH of the mineral soils ranged from 4.09 to 5.60 and of the mineral–organic soils from 7.38 to 7.76 (Table 4).

Table 4. Soil pH and peroxidase activity in mineral (1, 2) and mineral–organic (3, 4) soils in 2008

Sampling site	Sampling date									Range
	26.03	22.04	27.05	23.06	28.07	22.08	29.09	28.10	25.11	
	pH (KCl)									
1	4.94	4.79	5.50	5.60	5.32	4.69	5.58	5.47	5.55	4.69–5.69
2	4.61	4.70	4.09	4.95	4.79	4.10	4.55	4.67	4.42	4.10–4.95
3	7.57	7.50	7.45	7.55	7.45	7.44	7.43	7.47	7.38	7.38–7.57
4	7.60	7.57	7.50	7.76	7.63	7.50	7.48	7.53	7.62	7.48–7.76
	Peroxidase activity, nmol h^{-1} g^{-1}									$\bar{x} \pm \Delta X$
1	0.42±0.02	0.41±0.02	0.20±0.01	0.27±0.02	0.21±0.01	0.19±0.01	0.24±0.01	0.35±0.01	0.14±0.01	0.27±0.08
2	0.43±0.01	0.55±0.02	0.34±0.02	0.32±0.01	0.34±0.01	0.37±0.01	0.40±0.02	0.36±0.02	0.29±0.02	0.38±0.06
3	0.25±0.01	0.15±0.01	0.14±0.01	0.30±0.01	0.13±0.02	0.19±0.02	0.28±0.02	0.11±0.06	0.09±0.01	0.18±0.06
4	0.29±0.01	0.24±0.01	0.18±0.02	0.33±0.01	0.37±0.02	0.22±0.02	0.27±0.01	0.20±0.01	0.15±0.01	0.25±0.06

$\bar{x} \pm \Delta X$ – confidence interval of average at confidence level $\alpha = 0.05$ for $n - 1$ degrees of freedom.

These studies indicated that the flow of groundwater was accompanied by an increase of peroxidase activity from 2% to 51% (from site 1 to 2) in mineral soils in all periods of sampling. Peroxidase activity ranged from 0.14 to 0.55 nmol h^{-1} g^{-1} in mineral soils (Table 4). A similar trend was observed in mineral–organic soils: peroxidase activity increased from site 3 to 4, ranging from 11% to 46% or from 0.09 to 0.37 nmol h^{-1} g^{-1}. However, no significant differences were observed between the yearly mean of peroxidase activities of the two kinds of soils. Peroxidase reacts non-selectively via free-radical mechanisms, using hydrogen peroxide as an electron acceptor in the reactions. As is well known, the role of enzymes in coupling reactions leading to polymerization is limited to the oxidation of the substrates. The abundance of relatively stable peroxidases undoubtedly has a role in the synthesis of macromolecules such as HA. Moreover, the polymerization of readily degradable litter, soil organic matter, and nitrogen-containing compounds to higher-molecular-weight persistent organic compounds such as humic and fulvic acids influences the long-term storage of carbon in soils and the biological availability of soil nitrogen (Kerstetter et al., 1998; Dec et al., 2001).

Our results indicated an increase of the concentrations of Fe(III) ions (by 1–29%), Fe(II) ions (12–42%), and total iron (6–36%) in mineral soils accompanying the flow of groundwater during most periods of sampling (Table 5). The concentrations of total iron ranged from 7.09 to 18.27 mg kg^{-1} in the mineral soils. However, in the mineral–organic soils the concentrations of all iron forms showed a decreasing trend accompanying the flow of groundwater in most periods of sampling. The concentration of Fe(II) ions fell by 11–42%, that of Fe(III) by 4–48%, and of total iron by 8–45%. In mineral–organic soils the content of total iron ranged from 3.78 to 12.73 mg kg^{-1} (Table 5).

Reuter & Bell (2001) reported that ferrous iron is far more soluble than ferric iron, thus creating an increase in Fe mobility. Fe(II) can be transported within soils and landscapes via soil solution along redox gradients. According to Lovley & Anderson (2000), dissimilar Fe(III) reduction significantly influences the fate of both organic and inorganic compounds in pristine as well as contaminated subsurface environments. In deep pristine aquifers Fe(III) reduction can be an important processes for the oxidation of organic matter, increasing the concentrations of dissolved inorganic C and dissolved Fe(II), at the same time preventing the accumulation of sulphide.

It seems that the complexation of Fe with dissolved organic matter plays a fundamental part in acidification and pedogenesis and prevents immobilization by precipitation like inorganic metal complexes (Jansen et al. 2002, 2003; Grybos et al., 2007). Our research showed that the DOC concentration ranged from 0.83 to 1.65 g kg^{-1} in mineral soils and from 0.83 to 1.46 g kg^{-1} in mineral–organic soils (Table 6).

The lower ratios of DOC/total iron (66–199) and DOC/Fe(III) (127–339) in the mineral soils than in the mineral–organic soils (76–275 and 147–571, respectively) high concentrations of the forms of Fe in these complexes.

Table 5. Iron(II), iron(III), total iron, and iron(II)/iron(III) in mineral (1, 2) and mineral–organic (3, 4) soils in 2008

Sampling site	Sampling date									$\bar{x} \pm \Delta X$
	26.03	22.04	27.05	23.06	28.07	22.08	29.09	28.10	25.11	
	Fe(II), mg kg^{-1}									
1	4.80±0.13	4.36±0.11	3.28±0.15	6.66±0.18	3.99±0.14	3.11±0.15	3.68±0.12	3.48±0.17	6.02±0.11	4.38±0.95
2	3.79±0.12	5.04±0.15	2.67±0.13	6.49±0.11	3.58±0.15	3.58±0.10	3.72±0.09	4.93±0.13	8.45±0.18	4.69±1.38
3	4.80±0.14	4.83±0.10	2.91±0.19	3.72±0.11	3.75±0.17	5.41±0.12	4.33±0.13	3.45±0.11	6.32±0.11	4.39±0.81
4	2.77±0.11	5.21±0.13	1.96±0.12	4.06±0.10	2.54±0.14	3.75±0.16	3.85±0.10	3.75±0.14	4.46±0.11	3.59±0.77
	Fe(III), mg kg^{-1}									
1	4.50±0.09	5.44±0.17	4.04±0.13	8.39±0.12	4.53±0.13	3.98±0.12	4.01±0.12	3.71±0.13	7.11±0.15	5.08±1.25
2	6.41±0.15	7.20±0.12	5.62±0.13	9.52±0.11	5.32±0.10	6.14±0.09	5.84±0.15	6.38±0.14	9.82±0.16	6.92±1.26
3	5.11±0.14	5.35±0.09	3.10±0.12	5.14±0.19	4.07±0.15	5.20±0.15	4.32±0.15	3.53±0.10	6.41±0.14	4.69±0.78
4	2.64±0.15	5.65±0.13	1.82±0.17	3.44±0.13	2.83±0.14	3.37±0.10	4.16±0.16	4.10±0.14	4.35±0.12	3.60±0.86
	Total iron, mg kg^{-1}									
1	9.30±0.13	9.80±0.17	7.32±0.15	15.05±0.13	8.52±0.12	7.09±0.18	7.69±0.13	7.19±0.15	13.13±0.10	9.45±2.17
2	10.20±0.12	12.24±0.16	8.29±0.10	16.01±0.13	8.90±0.11	9.72±0.11	9.56±0.08	11.31±0.11	18.27±0.13	11.61±2.61
3	9.91±0.11	10.81±0.15	6.01±0.10	8.86±0.11	7.82±0.14	10.61±0.12	8.65±0.14	6.89±0.15	12.73±0.10	9.07±1.57
4	5.41±0.16	10.86±0.12	3.78±0.12	7.48±0.10	5.37±0.11	7.12±0.11	8.01±0.12	7.85±0.17	8.81±0.11	7.19±1.59
	Fe(II)/Fe(III)									
1	1.07	0.80	0.81	0.79	0.88	0.78	0.92	0.94	0.85	0.87±0.07
2	0.59	0.70	0.48	0.68	0.67	0.58	0.64	0.77	0.86	0.66±0.08
3	0.94	0.90	0.94	0.72	0.92	1.04	1.00	0.98	0.99	0.94±0.07
4	1.05	0.92	1.08	1.18	0.90	1.11	0.93	0.92	1.03	1.01±0.07

$\bar{x} \pm \Delta X$ – confidence interval of average at confidence leve $\alpha = 0.05$ for $n - 1$ degrees of freedom.

Table 6. Dissolved organic carbon (DOC) and total organic carbon (TOC) in mineral (1, 2) and mineral–organic (3, 4) soils in 2008

Sampling site	Date of sampling									$\bar{x} \pm \Delta X$
	26.03	22.04	27.05	23.06	28.07	22.08	29.09	28.10	25.11	
	DOC, g kg^{-1}									
1	1.01±0.06	1.24±0.08	1.13±0.07	0.99±0.06	1.22±0.06	1.35±0.08	0.93±0.08	1.19±0.06	1.03±0.04	1.12±0.10
2	1.02±0.04	0.97±0.06	1.65±0.06	1.21±0.07	0.83±0.04	1.63±0.07	1.08±0.06	1.15±0.08	1.27±0.08	1.20±0.21
3	1.15±0.05	1.00±0.04	0.97±0.08	1.46±0.08	0.86±0.08	1.01±0.07	0.95±0.08	1.04±0.04	1.28±0.06	0.97±0.14
4	1.06±0.04	0.83±0.06	1.04±0.06	1.18±0.06	0.92±0.04	0.93±0.08	1.03±0.06	0.93±0.05	1.00±0.06	0.99±0.05
	TOC, g kg^{-1}									
1	12.95±0.22	20.06±0.41	18.03±0.25	15.65±0.22	17.30±0.21	19.51±0.21	20.06±0.30	23.66±0.35	21.22±0.41	18.72±2.43
2	13.52±0.17	15.28±0.19	18.57±0.19	16.43±0.17	14.28±0.17	16.49±0.21	15.28±0.28	14.68±0.32	15.37±0.31	15.54±1.13
3	36.11±0.13	34.96±0.13	31.29±0.19	36.74±0.19	31.77±0.19	26.95±0.21	33.29±0.23	31.05±0.30	42.01±0.24	33.80±3.30
4	31.89±0.28	34.13±0.38	35.37±0.21	36.21±0.19	34.73±0.20	33.40±0.21	38.76±0.28	36.73±0.26	44.89±0.30	36.23±2.82

$\bar{x} \pm \Delta X$ – confidence interval of average at confidence level $\alpha = 0.05$ for $n - 1$ degrees of freedom.

Jansen et al. (2003) observed that due to the much higher fractions of soluble iron–DOM complexes in the Fe(II) than in the Fe(III) ion experiments, a change in redox potential increasing the relative contribution of Fe(II) leads to a strong rise in DOM mobility, which increases with increasing metal/organic carbon ratios.

It is known that the organic matter present in soils can be classified as either humic or non-humic material. Humic compounds are highly polymerized colloidal products of microbial decomposition of plant material. Although soil organic matter tends to form only a small percentage of the soil mass, it has a very great influence on soil chemical and physical properties, especially with regard to the behaviour of contaminants (Harrison, 1992). There are significant differences between the absorption spectra of the HA in mineral and mineral–organic soils (Szajdak et al., 2002b). In our previous work (Szajdak et al., 2002b) it was proved that the molecular structure of the HA from soils under a shelterbelt depends on the kind of soil and the direction of the flow of groundwater. The HA from mineral–organic soils are characterized by a higher degree of condensation and more extended polyconjugation systems in their molecules. In both soils, an increase in the direction of the flow of groundwater is accompanied by similar trends in the molecular structure of the HA. The changes in the molecular structure of HA were connected with the rise in the content of carbohydrates and carbonyl-containing groups and reduction of the degree of aromatic polyconjugation in their molecules, which reflects the lowering of the degree of humification or chemical maturity (Szajdak et al., 2002a, 2002b).

According to our present results, the TOC concentration ranged from 12.95 to 23.66 g kg^{-1} in mineral soils and from 26.95 to 44.89 g kg^{-1} in mineral–organic soils (Table 6), and the average yearly concentration of TOC was higher in mineral–organic soils than in mineral soils. A significant relationship was observed between TOC and peroxidase activity, total iron, and Fe(III) (Table 7). Organic matter is known to be redox reactive. The roles and effectiveness of specific functional groups in metal reduction are still subjects of intensive investigation. Humic substances could act as electron mediators or shuttles between microorganisms and Fe(III) oxide minerals. Certain anaerobic microorganisms may reduce organic matter (as an electron acceptor), which then donates electrons to reduce Fe(III)-containing minerals to release soluble Fe(II) (Cervantes et al., 2002; Chen et al., 2003).

Table 7. Significant linear correlations at the confidence level $\alpha = 0.05$ (mineral and mineral–organic soils, sampling sites 1, 2, 3, 4)

Relationship $y = f(x)$	Correlation coefficients
Peroxidase activity = f(Fe(II)/Fe(III))	−0.465
Peroxidase activity = f(TOC)	−0.558
Peroxidase activity = f(pH)	−0.571
Total iron = f(TOC)	−0.391
Total iron = f(pH)	−0.413
Fe(III) = f(TOC)	−0.436
Fe(III) = f(pH)	−0.475

The results of Scott et al. (1998) provide direct evidence that organic radicals in humic substances, which are primarily of the quinone group, are reduced when humics-reducing microorganisms transfer electrons to humic substances. These authors suggested that quinones, which are known as important electron-accepting groups in humic substances, may be dynamically involved in carbon in anaerobic environments. Moreover, Fe(III)-reducing microorganisms can transfer electrons derived from the oxidation of organic compounds and H_2 to humic substances. Thus, in anaerobic soils humic substances may serve to shuttle electrons from the surface of Fe(III)-reducing microorganisms to Fe(III) oxides (Scott et al., 1998).

According to Stevenson (1982), quinines are structural units and are very abundant in humus. Our previous results (Szajdak et al., 2002a, 2002b) agreed with those obtained by Scott et al. (1998). Electron spin resonance used in our investigations provided direct evidence that quinone moieties are functional groups accepting electrons during the microbial reduction of humus. Thus quinones are good analogues for the function of humus as a terminal electron acceptor. Utilization of ^{13}C NMR spectroscopy in our previous studies (Szajdak et al., 2002b) for dissolved HA indicated a predominant aliphatic character with a high content of alkyl C (30–43%) and *O,N*-substituted alkyl C (42–47%), and a very low content of aromatic C (4–17%). For HA from the mineral soil, a rise in the direction of the flow of groundwater leads to a rise in carbohydrate C (65–100 ppm) and C in carbonyl-containing groups, predominantly carboxyl (165–200 ppm). For HA from the mineral–organic soils, a reduction of C in methoxysubstituted phenols (from 150–152 to 55–57 ppm) and a significant rise in carbohydrate C accompanied the flow of groundwater.

The experimental results were treated statistically by the method of least squares at the confidence level $\alpha = 0.05$. Statistical analysis showed a significant relationship between peroxidase activity and Fe(II)/Fe(III), TOC, and pH and also total iron and TOC and pH, as well as Fe(III) and TOC and pH in mineral and mineral–organic soils (Table 7). However, no significant relationship was found for peroxidase activity and total iron or DOC.

Degradation and polymerization of organic compounds in soil are responsible for the formation of humic molecules, which are characterized by a complex polymeric structure with aromatic and aliphatic moieties (Tan, 2003). Processes such as mineralization of organic carbon or organic nitrogen to CO_2 and NH_4^+ can be carried out by a variety of microbial species. Urease catalyses the hydrolysis of urea with the release of ammonium and can contribute significantly to the cycling of soil nitrogen. Microbially mediated transformation of organic matter, and specifically nitrogen-containing functional groups, occurs during microbial respiration through which the acquisition of organic forms of nitrogen appears to be mediated by redox chemistry (Fimmen et al., 2007).

Our earlier investigations demonstrated a decrease of the content of total nitrogen and urease activity on the mineral soils of the investigated transect. The decrease of the contents of both agreed with the flow of groundwater during the entire vegetation season (Szajdak et al., 2002a; Meysner et al., 2006). However, the

present study indicated a decrease of the concentration of total iron and peroxidase activity in mineral soils while in mineral–organic soils the content of total nitrogen, urease activity, and peroxidase activity increased in the direction of the flow of groundwater.

Our previous investigations have shown for both kinds of soils that the flow of groundwater is accompanied by a decrease in the degree of humification or chemical maturity of HA (Szajdak et al., 2002b). The results presented in our paper suggest that this phenomenon may be due not only to the influence of the forest island and different conditions of humification but also to other effects, one of which may be the direction of oxidation and reduction processes in the sites located on both kinds of soils.

CONCLUSIONS

1. The study indicated an impact of the forest island located on mineral and mineral–organic soils on the changes of peroxidase activity and the total iron concentration accompanying the flow of groundwater. Peroxidases are known to be involved in the reactions that result in the polymerization of organic substances, increasing their environmental persistence.
2. Our results showed that the flow of groundwater was accompanied by an increase in the peroxidase activity and the concentrations of all forms of iron in the mineral soils during most periods of sampling.
3. In the mineral–organic soils TOC decreased while peroxidase activity and the concentrations of all forms of iron increased in the direction of the flow of groundwater during most periods of sampling. A decrease of TOC and an increase of peroxidase activity and all forms of iron were observed in most periods of sampling to accompany the flow of groundwater in the mineral-organic soils.

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