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## OXIDOREDUCTIVE ENZYMES ACTIVITY AS SECONDARY TRANSFORMATION INDEX OF PEAT-MOORSH SOILS

### AKTYWNOŚĆ ENZYMÓW OKSYDOREDUKCYJNYCH JAKO WSKAŹNIK WTÓRNEGO PRZEOBRAŻENIA GLEB TORFOWO-MURSZOWYCH

**Abstract:** Transformation of peat into moorsh in aerobic conditions after drainage causes changes in molecular structure and degradation of organic matter. Assessments of water retention and dewatering in peat indicate that agricultural activity has left a negative impact on peatland. Peat-moorsh samples were collected from three sites in the Wrzesnica River valley. Soil cores were subsequently divided into 0-25, 25-50, 50-75, 75-100 cm depth intervals. The following oxidoreductive enzymes activity was determined: phenol, xanthine and urate oxidase, peroxidase and nitrate reductase. In analyzed peat-moorsh samples have shown significant increase of phenol (1.3-2.3 times), xanthine (1.4-2.0 times) oxidase activity in all sites, and urate oxidase (1.4-2.5 times), peroxidase (1.6-3.8 times) activity in the first and the second sites within peat-moorsh profile. An opposite trend was recorded for nitrate reductase activity. The activity of this enzyme significant decreased with soil depth profile from 1.4 to 5.2 times. It was connected with degree of secondary transformation determined by the water holding capacity index ( $W_i$ ), porosity, moisture, the concentration of total phenolic, dissolved and total organic carbon. The investigations confirmed that enzymes activity is a good factor of the changes taking places in peat-moorsh soils. Oxidoreductive enzymes are important in catalyzing several pathways necessary for the stabilization of soil structure and organic matter formation.

**Keywords:** peat-moorsh soils, oxidoreductive enzymes, organic matter, secondary transformation index

### Introduction

Peat is characterized by colloidal behavior and irreversible loss of wettability produced by drying. Long-term cultivation and agricultural use of peatlands and their exploitation have revealed a number of effects including lowering of the table, increased aeration, changes in plant communities, and release of carbon content. The intensity of moorsh-forming process is shown by morphological and structural transformations, enrichment in humic substances, changes in mineral composition, biological and enzymatic activities [1, 2].

Soil enzymes are essential for catalyzing reactions necessary for organic matter decomposition and their activities are strongly influenced by the content of organic matter in the soil. They catalyze each phase of given substrates degradation leading to their decomposition. Most of these processes are initiated by oxidoreductive enzymes. The enzyme activities have often used as indicator of potential microbial activity. Temperature, pH, and nutrient supply have been reported as some of the controlling factors of the enzymatic processes in soils [3].

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In this paper, we present preliminary results which the aim was confirmed that oxidoreductive enzymes activity is a good the secondary transformation index of peat-moorsh profile. Research project is being performed.

### Materials and methods

Peat-moorsh samples were collected in the April 2018 year from three sites (about 100 m apart) along the Wrzesnica River valley in Goranin of west-central Poland (39 km east of Poznan, administrative district Czarniejewo, 52° 27' 28.372" N, 17° 30' 20.962" E). Soil cores were subsequently divided into 0-25, 25-50, 50-75, 75-100 cm depth intervals by taking of 10 independent peat-moorsh cores from each level using the Instorf sampler (Fig. 1). The soil material represented medium moorsh soil formed from alder swamp forest peat, strongly decomposition, underlain with lime gyttja (MtIIcc). Classification of peat was carried out according to standard PN-85/G-02500 Peat.



Fig. 1. Peat-moorsh cores collected by the Instorf sampler in Wrzesnica River valley (photo K. Styła)

The first site is an anthropogenic, wet meadow, bogginess, the structure of which is formed by the phytocenosis of the *Scirpetum sylvatici* community and additionally by *Carex gracilis*, and *Juncus articulatus*. There are 39 species of plants. At the second site there is a collection of high boggy vegetation with the dominance of *Iris pseudacorus*. Tested lobe is not a typical form of *Iridetum pseudacori* community, because it is characterized by a large share of meadow species. There are 38 species of plants. *Caricetum ripariae* community covers the banks of the watercourse as well as the small surface of the river valley at the third site. In the researched area *Carex rostrata* is dominant. It is one of the peat-forming communities, producing heavy, high-ash sedge peat. Phytocenosis creates 24 species of plants.

The samples were dried at 20 °C and sieved through 1 mm mesh and stage of secondary transformation, pH (in 1N KCl), porosity, total phenolic, total nitrogen ( $N_{total}$ ), ammonium ( $N-NH_4^+$ ) and nitrate ( $N-NO_3^-$ ) ions, dissolved organic carbon (DOC) and total

organic carbon (TOC) were determined. Stage of secondary transformation was determined according to Gawlik method [4]. Differentiation between the water holding capacity of peat-moorsh materials was expressed with the help of the  $W_1$  index calculated from the formula  $W_1 = b a^{-1}$ . The first (subsample *a*) was soaked for seven days and centrifuged at 1000 g. Then it was weighed, oven dried at 105 °C. The same procedure was applied to the second portion of the soil sample (subsample *b*). Part *b* after drying at room temperature before soaking was dried at 105 °C. Porosity was calculated from the bulk density ratio of the soil to the density of solids [5]. The concentration of total phenolics was measured colorimetrically at  $\lambda_{max} = 765$  nm [6]. Total nitrogen was evaluated by the Kjeldahl method. Ammonium ions were measured on ion chromatograph Waters 1515 (USA) and nitrate ions were determined on ion chromatograph HIC-6A (Shimadzu Japan) [7]. Dissolved organic carbon was evaluated on TOC 5050A (Shimadzu Japan). The total organic carbon was analyzed on Total Organic Carbon Analyzer (TOC 5050A) with Solid Sample Module SSM-5000A (Shimadzu Japan) [8]. In the top layer (0-25 cm) at the first, the second and the third sites measurements of redox potential Eh amounted -131.25, -76.36, -62.62 mV, respectively and values of temperature 9.0, 10.4, 6.9 °C, respectively.

For analyses of phenol, xanthine oxidase and nitrate reductase fresh peat-moorsh from each site were connected together to give the so-called average mixed sample. Phenol oxidase [EC 1.14.18.1] activity in peat-moorsh soil was determined colorimetrically at  $\lambda_{max} = 525$  nm by Perucci method using a UV-VIS spectrophotometer Beckman DU<sup>®</sup>-68 USA [9]. Peroxidase [EC 1.11.1.7] activity in peat-moorsh soil was measured colorimetrically at  $\lambda_{max} = 460$  nm by Bartha and Bordeleau method [9]. Urate oxidase [EC 1.7.3.3] was measured colorimetrically at  $\lambda_{max} = 293$  nm by Martin-Smith method [10]. Xanthine oxidase [EC 1.17.3.2] activity was assayed colorimetrically at  $\lambda_{max} = 290$  nm by Krawczyński method [9]. Nitrate reductase [EC 1.7.99.4] activity was estimated colorimetrically at  $\lambda_{max} = 520$  nm by Kandeler method [7, 9].

All chemical and biochemical analyses were run in triplicate, and the results were averaged. The confidence intervals were calculated using the following formula:  $\bar{x} \pm t_{\alpha(n-1)} SE$ , where:  $\bar{x}$  - mean;  $t_{\alpha(n-1)}$  - the value of the Student test for  $\alpha = 0.05$ ;  $n - 1$  - degree of freedom,  $SE$  - standard error. All the chemicals used in this study were of the analytical grade of purity.

## Results and discussion

Removal of water from peats is accompanied by an increase in aeration, considerable physicochemical changes of solid phase, and frequently leads to the breakdown of soil structural build-up. Transformation of peat into moorsh in aerobic conditions after drainage is called the secondary transformation of organic matter. The degree of the secondary transformation can be characterized by the water holding capacity index  $W_1$  [2]. The values of the  $W_1$  index were the highest in the top layer of 0-25 cm at the first site (0.813), in the layer of 0-25 and 25-50 cm at the second site (0.782 and 0.857, respectively) and the third sampling site (0.787 and 0.823, respectively) (Table 1). This part of peat-moorsh was classified as strongly secondary transformed. The values of  $W_1$  index decreased with depth and were classified as medium secondary transformed for the other levels of the peat-moorsh profile.

In the investigated peat-moorsh porosity values were lower in the layer of 0-25 cm at the first (84.04 %), the second (86.39 %), and the third sites (84.19 %) and increased with depth at all levels of peat-moorsh profile (Table 1). The soil differed greatly in their volumetric porosity, which was related undoubtedly to the degree of mucking and secondary transformation of the soil mass. It was reported by Inicki and Zeitz [1] that lower total porosity in muck was the effect of compaction caused by subsidence of peatland, changes in structure and transformation of organic matter. However, our studies confirmed that peat-moorsh samples moisture were lower at 0-25 cm and increased from 1.1 to 1.3 times with depth at all sites of peat-moorsh profile (Table 1). The significant criteria for predicting the capability of peat-moorsh soil to support microbial reactions are measurements of pH. The pH was strongly to slightly acidic at all sites of peat-moorsh sampling, and the highest values from 5.75 to 6.44 were measured at the first site. Investigation of total phenolic which is capable to accumulate of the lignin-humic complex within peat-moorsh profile has shown the significant increase in its content with depth from 1.3 to 1.6 times at all sites (Table 1).

Table 1

Physical parameters and content of total phenolic from three sites in Wrzesnica River valley

Site	Depth [cm]	$W_1$	Class	Stage of secondary transformation	Porosity [%]	Moisture [%]	pH (KCl)	Total phenolic [mg g <sup>-1</sup> ]
1	0-25	0.813	IV	Strongly	84.04	55.30	6.44	0.07±0.01
	25-50	0.710	III	Medium	85.15	58.90	6.15	0.08±0.01
	50-75	0.726	III	Medium	87.01	69.98	6.14	0.09±0.01
	75-100	0.688	III	Medium	87.43	73.89	5.75	0.11±0.02
2	0-25	0.782	IV	Strongly	86.39	72.75	5.55	0.11±0.02
	25-50	0.857	IV	Strongly	86.77	71.30	5.34	0.14±0.02
	50-75	0.725	III	Medium	87.24	76.36	5.46	0.18±0.01
	75-100	0.704	III	Medium	87.36	80.89	5.83	0.18±0.02
3	0-25	0.787	IV	Strongly	84.19	61.92	6.07	0.09±0.01
	25-50	0.823	IV	Strongly	85.50	70.59	5.65	0.09±0.01
	50-75	0.748	III	Medium	87.17	77.48	5.69	0.10±0.01
	75-100	0.715	III	Medium	88.14	79.91	5.74	0.12±0.02

According to other authors [11, 12] a good of the changes in peat soil due to mucking is, among others, progressive differentiation of the total amino acid content. They were found the correlation between total amino acid content and degree of secondary transformation of peat soils. The nitrogen in the soil exists in organic and mineral form but organic connections are dominant and may give up to 90 % of total nitrogen content. The actual source of mineral nitrogen in soil is the degradation of organic matter. On the nitrogen changes influence temperature, humidity, oxygenation degree, granulometric composition and biochemical factors connected with enzymatic activity. The amounts of total nitrogen ranged among 9.18 and 22.18 at all places of peat-moorsh sampling and were higher at the third site in 50-75 and 75-100 cm (Table 2). Our investigations have shown that the ammonium ions concentrations were from 1.40 to 7.70 mg kg<sup>-1</sup> in all levels of the peat-moorsh profile. This was reflected in higher these contents at the third site in 25-50, 50-75 and 75-100 cm. Additionally, nitrous ions contents ranged from 1.05 to 4.90 mg kg<sup>-1</sup> at all sites of peat-moorsh sampling (Table 2).

DOC can contribute significantly to the cycling of soil nutrients and it can be a substrate for microbial growth. This fraction of organic carbon is also connected with the movement of xenobiotics in soils, and it is suggested that degradation of DOC may be an important source of CO<sub>2</sub> [13, 14]. It was revealed that the concentration of DOC, in contrast to contents of TOC, significant decrease with the depth of the soil profile from 1.2 to 1.8 times in all investigated peat-moorsh samples (Table 2). With increasing soil depth of TOC concentrations significantly increased for the first site by 2.6 times, the second site by 1.6 times, and the third site by 1.5 times for a depth of 0-25 cm and 75-100 cm (Table 2). The data were consistent with studies of Kalisz et al. [4]. The relationship between carbon and nitrogen is important in hydrogenic soils, during primary humification and secondary transformation of organic matter in peat-moorsh forming process. The C/N ratio was from 13.49 to 19.50 for first site, from 12.99 to 18.17, for the second site, from 10.11 to 11.50 for the third site. The highest C/N ratio was found in the layer of 75-100 cm at the first and the second sites of peat-moorsh sampling (Table 2).

Table 2

Chemical parameters from three sites in Wrzesnica River valley

Site	Depth [cm]	N <sub>total</sub> [g kg <sup>-1</sup> ]	N-NH <sub>4</sub> <sup>+</sup> [mg kg <sup>-1</sup> ]	N-NO <sub>3</sub> <sup>-</sup> [mg kg <sup>-1</sup> ]	DOC [g kg <sup>-1</sup> ]	TOC [g kg <sup>-1</sup> ]	C/N ratio
1	0-25	9.18±0.96	2.45±0.17	2.48±0.11	4.03±0.31	123.9±7.1	13.49
	25-50	13.22±0.92	1.40±0.11	4.90±0.15	4.93±0.47	207.0±8.0	15.66
	50-75	19.26±0.81	1.45±0.13	2.80±0.12	4.78±0.37	319.2±9.0	16.57
	75-100	16.58±0.87	2.48±0.15	1.40±0.10	4.01±0.21	323.3±7.1	19.50
2	0-25	17.47±0.61	2.80±0.21	1.75±0.16	6.39±0.33	226.9±6.2	12.99
	25-50	18.14±0.71	3.15±0.30	1.45±0.11	5.94±0.41	286.8±7.8	15.81
	50-75	20.2±1.1	3.18±0.37	1.05±0.19	5.21±0.68	289.4±9.0	14.36
	75-100	18.82±0.94	3.50±0.41	1.75±0.18	3.53±0.20	360.8±8.9	18.17
3	0-25	13.22±0.80	3.15±0.17	3.15±0.13	5.39±0.21	148.5±5.9	11.23
	25-50	18.59±0.94	7.70±0.67	2.45±0.14	5.45±0.70	213.8±4.5	11.50
	50-75	21.5±1.1	6.48±0.87	1.40±0.10	5.29±0.63	217.3±6.3	10.11
	75-100	22.2±1.0	5.60±0.73	4.55±0.17	4.57±0.13	227.3±7.1	10.25

The direction of organic matter transformation of peat-moorsh soil, as well as the intensity of these changes is determined by activity of oxidative enzymes: phenol, xanthine, urate oxidase, peroxydase and nitrate reductase due to the potential they have in catalysing oxidation and reduction reactions and changes of nitrogen and carbon as basic components of organic matter of soil. Phenol oxidase is one of the few enzymes able to degrade recalcitrant phenolic materials as lignin to o-diphenols and o-quinones, participates in the formation of humic acids. Polyphenolics inhibit decomposition by binding to the reactive sites of extracellular enzymes and through the formation of phenolic complexes. The activity of extracellular phenol oxidases may, therefore, affect the retention of carbon in the soil environment directly via the breakdown of recalcitrant organic matter and extracellular hydrolase enzymes from phenolic inhibition [15, 16]. In all analyzed sites of peat-moorsh sampling, phenol oxidase activity ranged from 12.5 to 32.2 μmol h<sup>-1</sup> g<sup>-1</sup> (Table 3). Investigation of phenol oxidase activity within peat-moorsh profile has shown the significant increase in its activity with depth from 1.3 to 2.3 times at all sites (Table 3). It was in line with lower W<sub>1</sub> index, DOC and higher porosity, moisture, total phenolic and

TOC (Tables 1 and 2). Other the enzyme involved in the oxidation of high redox potential phenols and additionally of aromatic amines in the presence of hydrogen peroxide as an electron acceptor in the reactions is peroxidase. As is well known, the role of peroxidase in coupling reactions leading to polymerization is limited to the oxidation of the substrates. Their aggregate activity mediates key ecosystem functions of lignin degradation, humification, carbon mineralization, dissolved organic carbon export and the biological availability of soil nitrogen compounds [17]. Our results indicated that peroxidase activity was between 0.82 and 3.08 nmol h<sup>-1</sup> g<sup>-1</sup> at all sites of peat-moorsh sampling reaching significant higher values in deeper layers at the first and the third sites of peat-moorsh profile (Table 3). It was in line with lower W<sub>1</sub> index, DOC and higher porosity, moisture, total phenolic and TOC (Tables 1 and 2). In contrast this enzyme activity significant decrease with depth of 1.8 times at the second site (Table 3).

Table 3

Enzyme activities from three sites in Wrzesnica River valley

Site	Depth [cm]	Phenol oxidase [μmol h <sup>-1</sup> g <sup>-1</sup> ]	Peroxidase [nmol h <sup>-1</sup> g <sup>-1</sup> ]	Urate oxidase [μmol h <sup>-1</sup> g <sup>-1</sup> ]	Xanthine oxidase [μmol h <sup>-1</sup> g <sup>-1</sup> ]	Nitrate reductase [μg N 24 h <sup>-1</sup> g <sup>-1</sup> ]
1	0-25	17.3±1.5	0.82±0.09	22.4±1.3	4.82±0.89	0.15±0.02
	25-50	15.9±1.1	1.31±0.08	42.8±2.5	4.86±0.97	0.44±0.05
	50-75	20.0±1.2	2.23±0.05	42.2±2.0	6.23±0.75	0.38±0.06
	75-100	22.9±1.7	3.08 ±0.15	55.0±2.1	8.98±0.69	0.21±0.03
2	0-25	12.5±1.2	1.96±0.09	43.0±3.2	12.6±1.2	2.95±0.11
	25-50	21.0±1.7	2.27±0.08	32.7±2.2	11.4±1.2	1.47±0.09
	50-75	24.8±1.4	1.65±0.05	22.7±1.9	13.3±1.1	0.77±0.05
	75-100	28.2±1.4	1.28±0.08	19.8±1.0	17.6±1.1	0.57±0.03
3	0-25	20.9±1.2	1.49±0.07	28.8±1.9	7.09±0.87	3.03±0.15
	25-50	26.9±1.5	1.46±0.06	31.7±2.0	9.03±0.93	1.49±0.17
	50-75	32.2±1.8	1.94±0.09	36.7±2.1	11.32±0.97	1.51±0.11
	75-100	29.7±1.9	2.40±0.09	41.2±2.2	14.5±1.0	1.17±0.09

Urate and xanthine oxidase plays an important role in purine metabolism. Urate oxidase catalyzes the oxidation of uric acid to allantoin which is more soluble and easy to be excreted than the starting compound [18]. Ureides for allantoin in particular are nitrogen rich compounds derived from purine rings. The catabolism of purines, primarily from nucleic acids, results in xanthosine production which generates xanthine. The ureide pathway starts with the conversion of xanthine to uric acid releasing nitrogen in the form of ammonia. This process has an important role in nitrogen transport [19]. At the first and the third sites the lowest of urate oxidase activity was found in the top layer (22.4 and 28.8 μmol h<sup>-1</sup> g<sup>-1</sup>, respectively) reaching significant higher values in 75-100 cm depth (55.0 and 41.2 μmol h<sup>-1</sup> g<sup>-1</sup>, respectively) (Table 3). It was in line with lower W<sub>1</sub> index, DOC and higher porosity, moisture, total phenolic, and TOC (Tables 1 and 2). In contrast, this enzyme activity significant decrease with depth of 2.2 times at the second site similar to peroxidase activity (Table 3).

Xanthine oxidase participates in the degradation of purine derivatives from nucleic acids and is assumed to be a rate-limiting step in purine metabolism. This enzyme oxidizes hypoxanthine and xanthine to uric acid in the purine catabolic pathway and participating in the cycle of nitrogen in soils [20]. Xanthine oxidase activity ranged from 4.82 to

17.6  $\mu\text{mol h}^{-1} \text{g}^{-1}$  in all sites of peat-moorsh profile and was the highest in 75-100 cm depth of the second site (Table 3). Investigation of xanthine oxidase activity within peat-moorsh profile has shown significantly increase in its activity with depth from 1.4 to 2.0 times at all sites (Table 3). It was in line with decrease  $W_1$  index, DOC and increase porosity, moisture, total phenolic and TOC (Tables 1 and 2).

Denitrification is known to be an important nitrogen cycle process wherever oxygen is limiting and carbon and  $\text{NO}_3^-$  are available. Among the most important enzymes involved in this process leading to gaseous products of  $\text{N}_2\text{O}$  and  $\text{N}_2$  is nitrate reductase. In the anaerobic conditions, nitrate ions are reduced to nitrite ions and nitrate reductase is the catalyst of this conversion [7, 21]. These investigations documented that this enzyme activity participating in the denitrification process ranged from 0.15 to 3.03  $\mu\text{g N } 24 \text{ h}^{-1} \text{g}^{-1}$  and significant decrease with depth from 1.4 to 5.2 times at all sites of peat-moorsh sampling (Table 3). According to Deiglmayra et al. [22] major factors controlling nitrate reductase activity were total organic carbon content and nitrate availability. Our studies were noted that porosity, moisture, total phenolic, TOC increased but  $W_1$  index and DOC decreased similarly to this enzyme activity with peat-moorsh depth (Tables 1 and 2).

## Conclusions

The study was conducted at different stage of secondary transformation in peat-moorsh profile and confirmed that oxidative enzymes activity is a good factor of the changes taking places in peat-moorsh soils. It was connected with the concentration of total and dissolved organic carbon, total phenolic, porosity, moisture, and  $W_1$  index.

In analyzed peat-moorsh samples have shown the significant increase of phenol and xanthine oxidase activity in all sites, urate oxidase and peroxidase activity in the first and the third sites within peat-moorsh profile at the lower stage of secondary transformation. An opposite trend was recorded for nitrate reductase activity. Nitrate reductase activity significant decrease at all sites of peat-moorsh sampling classified as medium secondary transformed.

These results indicated that the lower stage of secondary transformation was found to be more effective for the degradation of purine basis, peptides, phenolics compound and in the formation of organic matter.

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## AKTYWNOŚĆ ENZYMÓW OKSYDOREDUKCYJNYCH JAKO WSKAŹNIK WTÓRNEGO PRZEOBRAŻENIA GLEB TORFOWO-MURSZOWYCH

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**Abstrakt:** Zabiegi melioracyjne oraz eksploatacja złóż torfowych powodują zmiany w strukturze molekularnej i degradację materii organicznej. Długotrwała działalność rolnicza na odwodnionych glebach torfowych prowadzi do ich degradacji. Próbkę gleb torfowo-murszowych zostały pobrane z trzech stanowisk zlokalizowanych w dolinie rzeki Wrześnicy koło Czarniejewa z głębokości 0-25, 25-50, 50-75, 75-100 cm. W próbkach glebowych oznaczono aktywność enzymów oksydoredukcyjnych: oksydazy fenolowej, ksantynowej i moczanowej oraz peroksydazy i reduktazy azotanowej. Wykazano istotny wzrost aktywności oksydazy fenolowej (1,3-2,3 razy), ksantynowej (1,4-2,0 razy) przy wszystkich stanowiskach oraz oksydazy moczanowej (1,4-2,5 razy) i peroksydazy (1,6-3,8 razy) przy stanowisku pierwszym i trzecim wraz z głębokością profilu glebowego. Przeciwny trend odnotowano dla aktywności reduktazy azotanowej. Aktywność tego enzymu zmniejszała się istotnie wraz z głębokością profilu glebowego od 1,4 do 5,2 razy. Zmiany te były zgodne ze stopniem wtórnego przeobrażenia utworów torfowo-murszowych określanego wartościami wskaźnika chłonności wodnej ( $W_1$ ), porowatością, wilgotnością, koncentracją związków fenolowych, rozpuszczonego i ogólnego węgla organicznego. Badania potwierdziły, że aktywność enzymów oksydoredukcyjnych jest dobrym wskaźnikiem zmian zachodzących w glebach torfowo-murszowych. Enzymy te odgrywają kluczową rolę w katalizowaniu wielu szlaków metabolicznych niezbędnych do stabilizacji struktury gleby i tworzenia materii organicznej.

**Słowa kluczowe:** gleby torfowo-murszowe, enzymy oksydoredukcyjne, materia organiczna, wskaźnik wtórnego przeobrażenia