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OnLine First

Microbial enzymatic activities in soils of Vojvodina, Serbia: insights into the relationship with chemical soil properties

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Abstract: For an agricultural region such as the Vojvodina Province in northern Serbia, soil quality monitoring is very important. Enzymatic activities are proposed as good indicators as they respond to even the slightest changes in the soil. This study aimed to analyze the enzymatic activity levels across three different soil types in Vojvodina and to examine their connection to soil chemical properties and land use. All soil types (chernozem, vertisol, solonchak) were sampled at nine locations, each with 3 field plots. The activities of acid and alkaline phosphatase, β-glucosidase, dehydrogenase, and catalase were measured in samples, as well as the selected chemical properties. Results showed differences in enzymatic activity across different soil types and land use. The most active enzymes in vertisol were acid phosphatase and β-glucosidase; in solonchak, it was alkaline phosphatase; in chernozem, it was dehydrogenase. A high correlation between enzymatic activities and certain soil chemical properties (pH reaction, organic matter, organic carbon, total nitrogen) was also observed, underlining the existence of a relationship between different soil components. The highest determined correlation was between acid phosphatase and pH (r=-0.7), alkaline phosphatase and total nitrogen (r=0.7), and organic matter (r=0.72); the obtained correlations were found to be statistically significant.

Keywords: microbial enzymes; soil chemical properties; agricultural soil; soil quality; land use

INTRODUCTION

Soil quality is a multifaceted concept, typically characterized by physical, chemical, and biological attributes [1]. Microorganisms, a pivotal component of the soil's biological ecosystem, play a vital role in both soil formation, one of the factors in pedogenesis, and soil fertility. This is the result of microorganisms having substantial roles in the soil, including the decomposition of soil organic matter and nutrient cycling [2]. Characteristic microbial communities are present in each soil type. Cultivation practices often disturb Relationships inside soil communities that lead to the reduced number and enzymatic activities of microorganisms [3]. Chemical analyses of soil can provide valuable insights into soil fertility [4]. However, soil chemical properties are not the sole indicators of changes in soil, as they typically only alter significantly under extreme conditions. The study of biological and biochemical properties is also invaluable [5]. The use of biological indicators for soil quality is highly complex and remains a subject of an ongoing study [6]. Biochemical soil properties, such as soil enzymes, are good indicators of soil health and fertility, as they are involved in important metabolic processes and are responsive to even the slightest changes in soil [7-9]. Hence, when soil disturbances affect the microbial community, changes in enzymatic activities

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are also expected [10]. It is essential to study the factors influencing soil enzymatic activities [11]. Land use affects the soil organic matter content, which causes changes in soil chemical properties, further affecting soil microorganisms [7,12]. Different land use types, such as agricultural and undisturbed soils (e.g., forests, pastures), are characterized by different chemical property values and, consequently, different microbial community composition and enzymatic activity levels.

Acid and alkaline phosphatase, β-glucosidase, dehydrogenase, and catalase are among the most studied soil enzymatic activities. Phosphatases are a group of enzymes capable of hydrolyzing phosphoric acid. These enzymes are correlated with phosphorus stress, as they have substantial roles in P cycling [13,14]. β-glucosidase is a very common and predominant enzyme in soils, with a crucial role in the catalysis of β-glucosides, producing glucose, an important carbon and energy source [14,15]. Dehydrogenase transfers protons and electrons from substrates to acceptors, thus oxidizing soil organic matter. These processes are involved in soil microorganism respiration pathways and are considered to be related to soil type and air-water conditions [14,16,17]. Catalase prevents cell damage caused by reactive oxygen species (ROS) by splitting hydrogen peroxide into molecular oxygen and water [18]. The activity of catalase is related to aerobic organisms' metabolic activity and is studied as the soil fertility indicator [19].

The Autonomous Province of Vojvodina is mainly an agricultural region. Understanding the soil quality of such a region is important for enhancing agricultural productivity. Evaluating the soil's physical, chemical, biological, and biochemical properties is crucial in determining its overall quality, indicating a need to study these properties. Dominant soil types in Vojvodina are chernozem and vertisol. In the Vojvodina Province, chernozem and hydromorphic black soil cover more than 75% of the total area and are not distributed uniformly [20]. On the other hand, solonchak soil, due to its unfavorable water and physical properties, is not suitable for intensive agricultural production. However, it can be utilized for extensive natural pastures [21]. These soil types exhibit varying degrees of agricultural output, with chernozem and vertisol displaying high productivity with none or some restrictions, respectively, and solonchak showcasing relatively low agricultural potential with serious limitations. Saline soils are invaluable for the unique endemic ecosystems they support. These soils offer specialized habitats for a diverse range of highly specialized plants, animals, and other organisms [22].

The primary objective of this study was to assess the enzymatic activity levels in three distinct soil types in Vojvodina and to discern between variations in these activities in the different soil types. Additionally, the study aimed to establish connections between enzymatic activities, soil chemical properties, and land use.

MATERIALS AND METHODS
Study area
The study was conducted in the Autonomous Province of Vojvodina, northern Serbia. Vojvodina is characterized by diverse soils, including chernozem, vertisol, and solonchak [20]. These soil types were selected for investigation due to their prevalence in the region, as well as their specific characteristics. The identification of each soil type was conducted in two steps. Initially, it referenced Vojvodina's pedological map, a ratio of 1:50,000 [23]. Subsequently, the process included field and laboratory observations for each location, allowing for the determination of the soil group based on the WRB classification [24]. For each soil type, 3 locations were selected for sampling. At each location, soil samples were collected from 3 plots, 2 agricultural and 1 uncultivated control plot (Supplementary Table S1, Supplementary Fig. S1). Control plots were selected based on their immediate proximity and similar characteristics to agricultural plots of the same soil type. These plots were under minimal

anthropogenic influence during longer periods (forests, meadows, etc.). Chernozem was sampled in the region of Bačka (Rimski Šančevi, and Futog – field plots C1-C9), vertisol in the regions of Bačka and Banat (Bečej, Mužlja, and Vršac – field plots V1-V9), and solonchak in the region of Bačka (Rančevo, Kljajićevo, and Trešnjevac – field plots S1-S9). Sampling locations were chosen based on known typical soil chemical characteristics to obtain representative samples for each soil type.

Soil sampling

Soil sampling was conducted using the circular reference sample method [25]. Representative sampling locations were selected through a field survey. The center of each circle was recorded using a GPS device, and individual samples were taken according to a predetermined arrangement of points relative to the positioned center. A total of 20-25 individual samples were collected from each position, and an average composite sample was created, representing the investigated area. Five average composite samples (5 repetitions) were obtained from each investigated location. Sampling was performed using an agrochemical probe reaching a soil depth of 30 cm. This method is commonly used for monitoring soil nutrient content and changes in its properties. The sampling equipment was thoroughly washed with distilled water, sprayed with ethanol, and sterilized using a flame to reduce the risk of sample contamination. Soil samples were packed in sterile bags, transported to the laboratory at 4°C, and analyzed within the next 24 h.

Soil chemical properties analyses

To analyze soil chemical properties, soil samples were air-dried and subsequently ground in a soil mill to a particle size of <2 mm [26]. The pH value was determined potentiometrically in a suspension with water (active acidity) and 1 M KCl (exchangeable acidity) [27]. The determination of free $CaCO_3$ was performed volumetrically using a Scheibler calcimeter [28]. Organic matter (OM) content was determined using a modified Tyurin method based on the oxidation of soil organic carbon (SOC) with K_2CrO_7 [29]. The total nitrogen content was determined using CHNS elemental analysis on a VarioEL III analyzer [30]. Total organic carbon (TOC) content was also determined using CHNS elemental analysis on a VarioEL III analyzer according to the ISO procedure, 10694:2005 determination of organic and total carbon after dry combustion [31]. Readily available phosphorus P (AL) in the soil was determined by extraction of ammonium lactate [32], whereby detection was performed spectrophotometrically at the wavelength of 830 nm in a UV/VIS spectrophotometer using the phospho-molybdate-blue-method [33]. Readily available potassium K (AL) in the soil was determined by extraction of ammonium lactate [32] using a flame photometer. Soil dry matter (dm) content was determined by drying 1 g of soil at 105°C until a constant weight was achieved.

Soil enzymatic activities analyses

The activities of soil dehydrogenase, acid and alkaline phosphatase, β-glucosidase, and catalase were measured. Dehydrogenase activity was determined in 1 g of soil using 2,3,5-triphenyl tetrazolium chloride as substrate and incubating in tris(hydroxymethyl)aminomethane buffer (pH 7.6) for 24 h at 25°C [30-33]. Enzyme activity was expressed as µg triphenyl formazan (TPF) g^{-1} dm of soil h^{-1}. Acid and alkaline phosphatase activities were determined in 0.5 g of soil using p-nitrophenyl phosphate as substrate, incubating in a modified universal buffer (MUB), pH 6.5 for acid, and pH 11 for alkaline phosphatase) for 1 h at 37°C [13,38]. Enzyme activity was expressed as µg p-nitrophenol (pNP) g^{-1} dm of soil h^{-1}. The activity of β-glucosidase was determined in 0.5 g of soil using p-nitrophenyl-β-D-glucopyranoside as substrate, incubating in MUB for 1 h at 37°C [15,39]. Enzyme activity was expressed as µg pNP g^{-1} dm of soil h^{-1}. Catalase activity was determined by permanganometric titration after incubation of 2 g of soil with 0.3% hydrogen peroxide solution [40,41]. Enzyme activity was expressed as mL 0.2 M $KMnO_4$ g^{-1} dm of soil h^{-1}.

Statistical analysis

All statistical analyses were conducted in R Studio [42]. The *dplyr* package [43] was used for data manipulation. Determination of Pearson's correlation coefficient between soil chemical characteristics and enzymatic activities was performed using the *core* package [44]. The corrplot package was used to determine the statistical significance of the obtained correlations [45]. The Kruskal-Wallis test was used to determine differences in chemical properties and enzymatic activities between different soil types, and post-hoc analysis based on the Dunn test was conducted to determine differences between any two soil types. Visualization of all results was performed using the *ggplot2* package [46].

RESULTS

Soil chemical properties

The following soil chemical properties were examined as follows: pH in KCl, pH in H_2O, $CaCO_3$ content, OM, total nitrogen, and SOC content, and the amount of P_2O_5 and K_2O accessible to plants. The results are presented in Table 1 and Supplementary Fig. S2. The highest pH in KCl and H_2O was measured in solonchak soil samples, with mean values of 7.95 and 8.98, respectively. The pH of the chernozem soil samples was generally neutral, except for plot C3, which had a pH value below 7. Vertisol samples displayed lower pH, with the mean value of 5.30 in KCl, and 6.39 in H_2O. The pH in KCl of solonchak soil samples was significantly higher than that of chernozem and vertisol; the pH in H2O of solonchak was significantly higher than that of vertisol. The solonchak samples had the highest $CaCO_3$ content, with an average value of 20.16%, while the content in the vertisol samples was significantly lower, with a mean value of 0.32%. The OM content was similar across all three soil types, with the mean values around 2% and no significant differences between different soils. Organic carbon and total nitrogen contents were also similar in all three soil types. No significant differences were observed in P_2O_5 content in chernozem, vertisol, and solonchak. The content of K_2O was significantly higher in vertisol (mean value 35.06 mg/100 g) than in solonchak (mean value 17.44 mg/100 g).

Soil enzymatic activities

The analysis of acid and alkaline phosphatase, β-glucosidase, dehydrogenase, and catalase activity was conducted (Table 2, Fig. 1, Supplementary Fig. S3). Vertisol exhibited the highest acid phosphatase activity (mean value 55.58 µg *p*NP g^{-1} dm h^{-1}), which was significantly higher than activity in chernozem and solonchak. Accordingly, alkaline phosphatase activity was generally lower in all vertisol samples compared to acid phosphatase activity, with a mean value of 39.17 µg *p*NP g^{-1} dm h^{-1}. In chernozem, alkaline phosphatase activity with an average value of 36.76 µg *p*NP g^{-1} dm h^{-1} was higher than acid phosphatase activity with an average value of 19.10 µg *p*NP g^{-1} dm h^{-1}. The exception was plot C3, which exhibited elevated acid phosphatase activity. Similarly, alkaline phosphatase activity was generally higher in solonchak than acid phosphatase, with an average value of 46.25 µg *p*NP g^{-1} dm h^{-1}, except for plot S3, which represented the control soil. The activities of β-glucosidase and catalase were not found to be significantly different across the investigated soil types. Dehydrogenase activity was significantly lower in vertisol (mean value of 0.06 µg TPF g^{-1} dm h^{-1}) compared to chernozem (mean value of 0.79 µg TPF g^{-1} dm h^{-1}) and solonchak (mean value of 0.54 µg TPF g^{-1} dm h^{-1}).

Patterns in enzymatic activities across land-use types were observed, namely differences between overall activity in agricultural and control soils in all three soil types (Supplementary Fig. S4); however, neither of these differences was found to be statistically significant.

Correlation between enzymatic activities and soil chemical properties

Linear correlation based on Pearson's correlation coefficient (r) was calculated to test if a relationship between the analyzed enzymatic activities and soil chemical properties exists and what is the nature of the relationship (Fig. 2). Acid phosphatase exhibited a high negative correlation with pH in KCl (r=-0.74 ***) and pH in H_2O (r=-0.70 ***), moderate negative correlation with $CaCO_3$ content (r=-0.46 ***), and moderate positive correlation with OM (r=0.43 **), total nitrogen (r=0.42 ***), and SOC (r=0.59 ***) contents. A high positive correlation was observed between alkaline phosphatase and OM (r=0.72 ***), total nitrogen (r=0.70 ***), and SOC (r=0.61 ***) contents. Dehydrogenase activity did not correlate highly or moderately with any of the chemical characteristics. However, a low correlation was observed with pH in KCl, pH in H2O, and K2O content. A moderate negative correlation was noted between β-glucosidase activity and pH in KCl (r=-0.51 ***), pH in H_2O (r=-0.54 ***), and the $CaCO_3$ content (r=-0.41 ***), and a moderate positive correlation was observed with OM (r=0.52 ***), nitrogen (r=0.51 ***), and SOC (r=0.48 ***) contents. Catalase activity exhibited a low positive correlation with OM, nitrogen, and SOC contents and a very low correlation with the remaining examined chemical properties.

DISCUSSION

The results obtained for chernozem in this study agree with previously reported values for chemical properties [47,48], except for a slightly acidic pH value observed in plot C3 and significantly higher P_2O_5 content in plots C1 and C7. Plot C3 represents a forest control plot characterized by high organic matter content, which undergoes degradation, leading to soil acidification. About 42% of the plots analyzed in our study were below the lower limit of optimum phosphorus supply, while 23% of the plots were below the limit regarding potassium supply, which was reported to be 15 mg/100 g of soil [49]. Vertisol, which is characterized by high clay content, has an unfavorable chemical composition. Depending on the $CaCO_3$ content, these soils can be slightly acidic to slightly alkaline [47]. Our research findings for vertisol agree with the expected value range (pH 5.8-8.1) [47] for the chemical properties of vertisol, with a slightly acidic pH reaction observed across all field plots, indicating a lower $CaCO_3$ content. We observed a higher P_2O_5 content in plots V7 and V9. On the other hand, solonchak exhibits poor physical properties, as the presence of salt determines its chemical properties. The chemical analysis results obtained in this study are typical for solonchak and fall into previously reported ranges for pH from 7.8 to 10.45 and OM of 1-2%, reaching up to 4% [47].

Amongst the most used and change-responsive indicators of soil quality and health are soil enzymes. Enzyme activities usually correlate with the SOC and total nitrogen content since a higher organic matter content stimulates enzyme synthesis [50]. Organic matter is substantial in establishing an optimal soil structure and regulating the water, air, and heat conditions within the soil [51], which, in turn, enhances the activity of microorganisms. Soil pH reaction also highly affects enzymatic activity, influencing the solubility of enzymes, substrates, and cofactors.

Phosphatase activity in soil is known to be influenced by several soil chemical properties, such as pH, total nitrogen, organic phosphorus, and SOC [50-52]. Specifically, acid phosphatase activity correlates negatively with soil pH, while alkaline phosphatase exhibits a statistically significant positive correlation with pH value. We observed the highest acid phosphatase activity in acidic vertisol, indicating a strong negative correlation with pH, while alkaline phosphatase exhibited a very low positive correlation with pH reaction. Besides vertisol, acid phosphatase activity was found to be higher in one field plot on generally pH-neutral chernozem. The plot in question (C3) had an acidic pH value, which can be attributed to the soil being under forest. Our results show that both acid and alkaline phosphatase display statistically significant moderate to high positive correlation with total nitrogen and SOC

contents in the soil. These findings underscore the interplay between soil chemical properties and phosphatase activities, offering valuable insights into nutrient cycling processes in soil ecosystems.

The research has revealed a significant correlation between β-glucosidase and soil pH value and total nitrogen content [52,54]. We observed that the enzyme's activity exhibits a significant negative correlation with pH, indicating a decrease in activity with the increase in pH value [55]. Soil salinity is also known to suppress β-glucosidase activity. Slightly lower levels of glucosidase activity were measured in solonchak, but not significantly lower compared to chernozem, which can be attributed to the fact that chernozem has a neutral to slightly alkaline pH reaction, leading to a decrease in enzyme activity. In contrast, the highest enzyme activity levels were recorded in vertisol, which is characterized by a slightly acidic pH reaction.

Dehydrogenase activity is closely linked to the soil pH reaction, exhibiting a positive correlation [56]. This study confirmed a statistically significant low positive correlation exists, while there is negligible or no correlation with other soil chemical parameters except the K_2O content. The optimum soil pH for dehydrogenase activity is considered to be between 6.6 and 7.8, while acidic conditions suppress enzymatic activity [57,58]. The lowest dehydrogenase activity in our samples was recorded in vertisol, which is expected given the acidic nature of this soil type.

Based on our results, catalase activity exhibited a significant positive correlation with total nitrogen content, which is in agreement with other studies [56]. Catalase activity is also known to be negatively correlated with pH value, which was negligible in our study, implying that pH does not affect the enzyme's activity.

Enzymatic activities are known to differ significantly amongst different land-use types [59]; however, the differences we found between agricultural and control soils in enzymatic activity levels were not statistically significant.

CONCLUSIONS

Investigations performed on different soil types in Vojvodina confirmed a strong statistically significant relationship between enzymatic activity and soil chemical properties. The main factors affecting enzymes are the soil pH reaction, total nitrogen, organic matter, and the organic carbon content. A significant high correlation exists between acid phosphatase and pH, as well as alkaline phosphatase and organic matter, total nitrogen, and organic carbon. Moderate correlation is shown between acid phosphatase and organic carbon, and β-glucosidase and pH, organic matter, and total nitrogen. Dehydrogenase and catalase activities did not exhibit a correlation with the chemical properties. In addition, differences in enzymatic activity levels across soil types were detected. Acid phosphatase and β-glucosidase displayed the highest activity in vertisol, while alkaline phosphatase was the most active in solonchak. Dehydrogenase and catalase showed similar activity levels in all soil types. Regarding land-use type, some differences between agricultural and uncultivated control soil were observed but were without statistical significance. We conclude that enzyme activities are suitable soil quality indicators. However, to acquire a more accurate estimation of soil quality, future research should include more soil physicochemical properties and microbial community composition analysis since it is known that all soil components are connected.

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Data availability: Data underlying the reported findings have been provided as raw datasets that are available here:
[https://www.serbiosoc.org.rs/NewUploads/Uploads/Kuzmanovic%20et%20al_Raw%20Dataset_1.pdf], and
[https://www.serbiosoc.org.rs/NewUploads/Uploads/Kuzmanovic%20et%20al_Raw%20Dataset_2.xlsx]

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Table 1. Descriptive statistic parameter values of major chemical properties of three different soil types in Vojvodina Province, Serbia. Means followed by a different letter indicate significant differences.

Soil type	Parameter	Chemical properties							
		pH in KCl	pH in H_2O	$CaCO_3$ %	Organic matter %	Total N %	Organic C %	P_2O_5 mg/100 g	K_2O mg/100 g
chernozem	Min.	4.98	6.06	0.27	1.88	0.15	1.01	1.88	9.60
	Max.	7.44	8.48	16.86	3.34	0.23	2.46	83.93	37.34
	Mean	6.78 a	7.64 ab	3.49 ab	2.44 a	0.18 a	1.66 a	28.40 a	20.72 ab
	St. dev.	0.80	0.76	5.58	0.49	0.03	0.43	27.26	9.12
	Sample var.	0.64	0.57	31.15	0.24	0.00	0.18	743.27	83.22
vertisol	Min.	4.68	5.75	0.00	1.65	0.14	1.48	3.04	14.56
	Max.	5.91	6.83	0.56	4.13	0.27	3.65	78.55	62.62
	Mean	5.30 a	6.39 a	0.32 a	2.89 a	0.21 a	2.57 a	25.48 a	35.06 a
	St. dev.	0.37	0.37	0.20	0.81	0.04	0.85	26.94	16.12
	Sample var.	0.14	0.13	0.04	0.66	0.00	0.73	725.97	259.70
solonchak	Min.	7.49	8.30	15.44	1.34	0.12	1.75	7.50	13.89
	Max.	8.90	10.22	27.87	3.61	0.25	3.15	19.18	24.82
	Mean	7.95 b	8.98 b	20.16 b	2.76 a	0.20 a	2.44 a	15.10 a	17.44 b
	St. dev.	0.53	0.74	4.40	0.75	0.04	0.48	4.02	3.27
	Sample var.	0.29	0.55	19.32	0.56	0.00	0.23	16.16	10.70

Table 2. Descriptive statistics parameter values of enzymatic activities of 3 different soil types in Vojvodina Province, Serbia. Acid and alkaline phosphatase, and β-glucosidase activity are expressed as µg pNP g^{-1} dm h^{-1}, dehydrogenase as µg TPF g^{-1} dm h^{-1}, and catalase as mL 0.2 M $KMnO_4$ g^{-1} dm h^{-1}. Means followed by a different letter indicate significant differences.

| Soil type	Parameter	Enzymatic activities				
		Acid phosphatase	Alkaline phosphatase	β-glucosidase	Dehydrogenase	Catalase
Chernozem	Min.	5.35	29.27	0.96	0.11	0.68
	Max.	56.78	57.25	12.05	2.89	2.02
	Mean	19.10 a	36.76 a	6.92 a	0.79 a	1.14 a
	St. dev.	16.60	9.39	3.01	0.95	0.54
	Sample var.	275.67	88.09	9.08	0.90	0.30
Vertisol	Min.	32.57	9.78	4.63	0.02	0.59
	Max.	86.86	98.99	18.26	0.16	2.36
	Mean	55.58 b	39.17 a	11.53 a	0.06 b	1.34 a
	St. dev.	17.71	26.68	4.87	0.05	0.66
	Sample var.	313.81	711.87	23.74	0.00	0.43
Solonchak	Min.	15.19	7.83	3.00	0.03	0.50
	Max.	39.15	67.62	9.87	2.14	2.25
	Mean	22.05 a	46.25 a	6.62 a	0.54 a	1.39 a
	St. dev.	7.44	17.83	2.66	0.63	0.66
	Sample var.	55.42	317.76	7.10	0.39	0.44
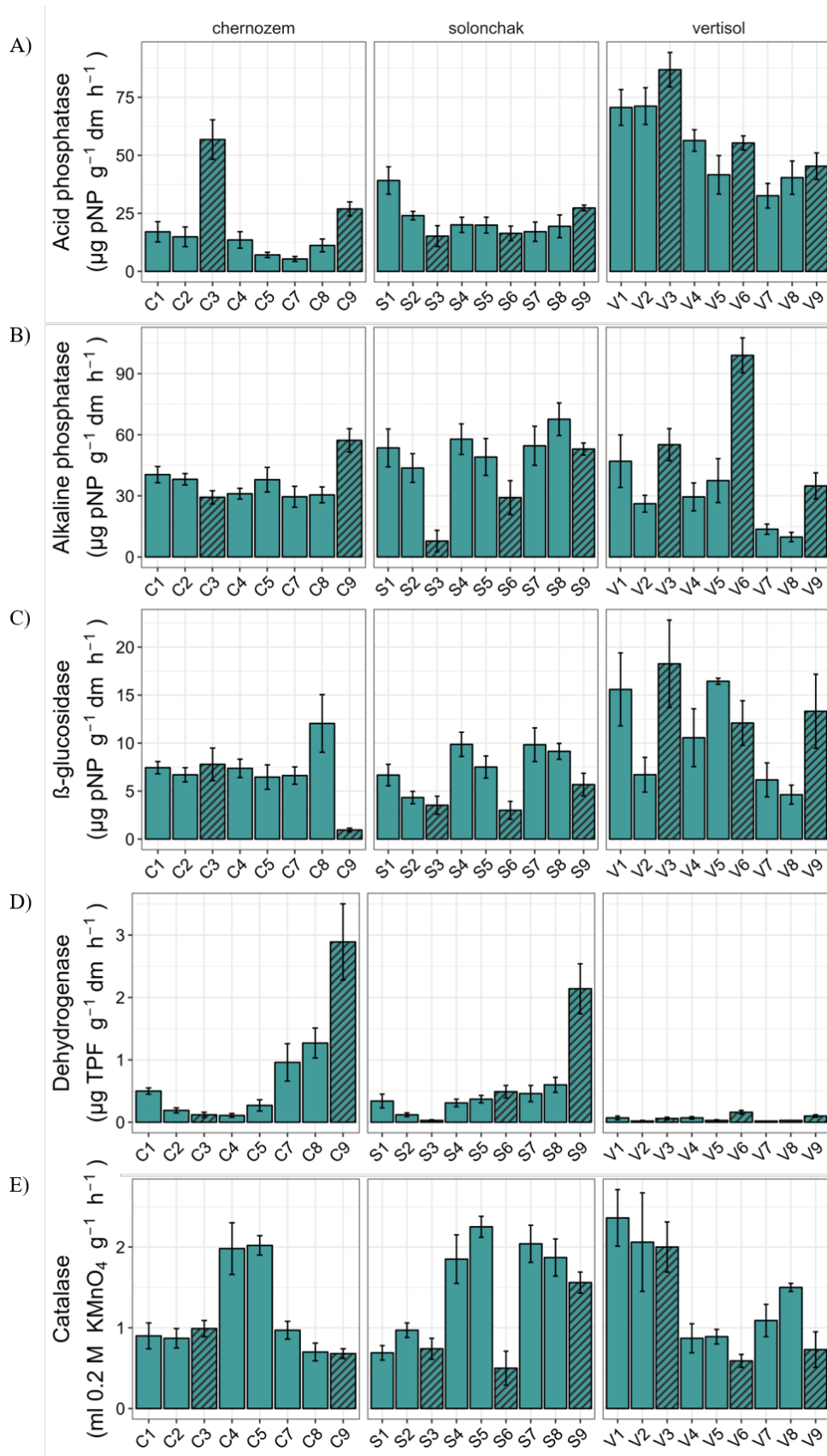
Fig. 1. Soil enzymatic activity levels. **A** – acid phosphatase, **B** – alkaline phosphatase, **C** – β-glucosidase, **D** – dehydrogenase, **E** – catalase. Control plots are striped (▨); field plot IDs are presented on the x-axis.
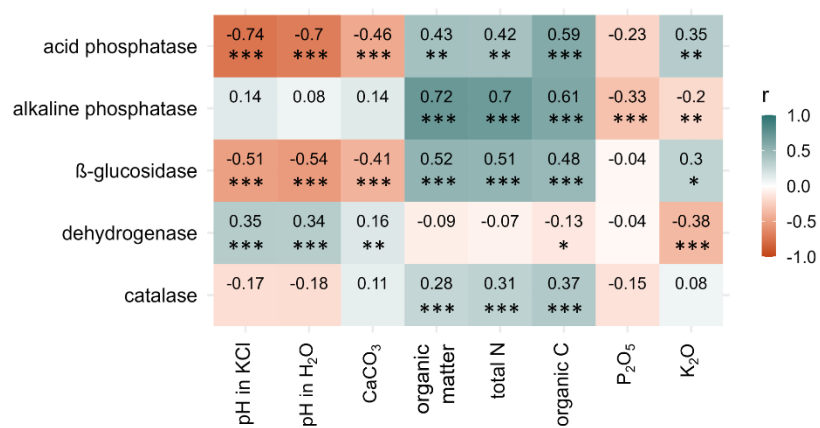
Fig. 2. Pearson's correlation between enzymatic activities and soil chemical properties (r). Statistical significance is presented with asterisks (* P≤0.05, ** P≤0.01, *** P≤0.001).

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Description of agricultural plots chosen for soil sampling, including location, soil type, brief plot description (land use, crop), and coordinates.

| Field plot ID	Location	Soil type	Plot description	Coordinates	
				Lat.	Lon.
C1	Rimski Šančevi 1	chernozem	agricultural, wheat	45.323533	19.845817
C2	Rimski Šančevi 1	chernozem	agricultural, corn	45.319593	19.918322
C3	Rimski Šančevi 1	chernozem	control, forest	45.326413	19.858557
C4	Rimski Šančevi 2	chernozem	agricultural, wheat	45.329126	19.845849
C5	Rimski Šančevi 2	chernozem	agricultural, wheat	45.324289	19.847339
C7	Futog	chernozem	agricultural, corn	45.245382	19.696786
C8	Futog	chernozem	agricultural, wheat	45.246626	19.696785
C9	Futog	chernozem	control, forest	45.240622	19.699131
V1	Bečej	vertisol	agricultural, orchard	45.583608	20.086958
V2	Bečej	vertisol	agricultural, sunflower	45.581293	20.086911
V3	Bečej	vertisol	control, meadow in the forest	45.579718	20.086729
V4	Mužlja	vertisol	agricultural, organic, wheat	45.289950	20.272072
V5	Mužlja	vertisol	agricultural, wheat	45.301575	20.275848
V6	Mužlja	vertisol	control, meadow	45.307951	20.258690
V7	Vršac	vertisol	agricultural, vineyard	45.105204	21.329008
V8	Vršac	vertisol	agricultural, pumpkin	45.103016	21.331516
V9	Vršac	vertisol	control, forest	45.105761	21.330247
S1	Rančevo	solonchak	agricultural, soybean	45.869438	19.125111
S2	Rančevo	solonchak	agricultural, corn	45.866464	19.126623
S3	Rančevo	solonchak	control, pasture	45.867231	19.121985
S4	Kljajićevo	solonchak	agricultural, wheat	45.736836	19.262121
S5	Kljajićevo	solonchak	agricultural, wheat	45.736168	19.264500
S6	Kljajićevo	solonchak	control, pasture	45.736830	19.264342
S7	Trešnjevac	solonchak	agricultural, corn	45.982334	20.000062
S8	Trešnjevac	solonchak	agricultural, wheat	45.981869	20.000192
S9	Trešnjevac	solonchak	control, pasture	45.984553	19.999160
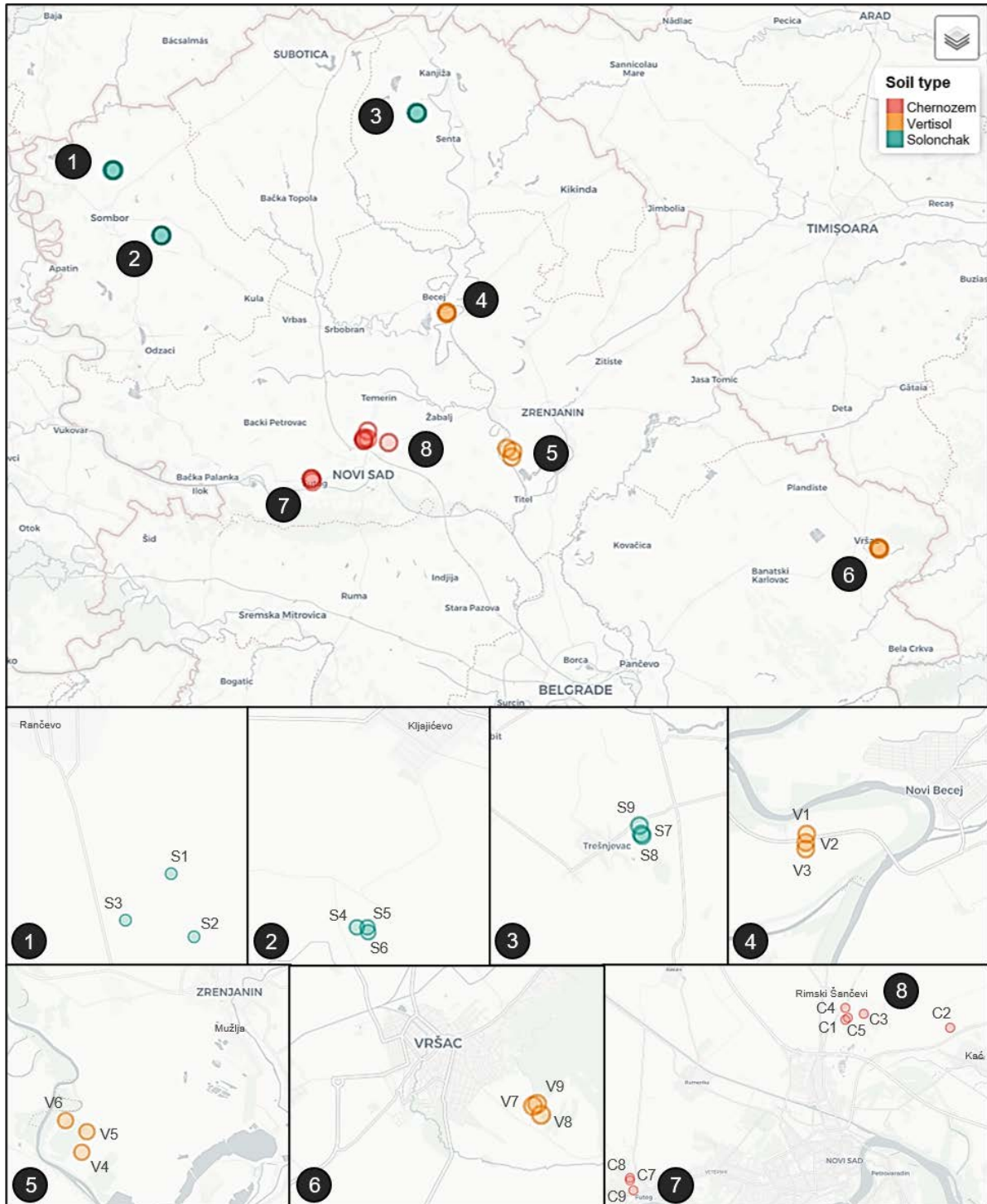
Supplementary Fig. S1. Soil sampling locations in the Autonomous Province of Vojvodina. Each soil type was sampled at 3 locations – solonchak (1-3): Rančevo (S1-S3), Kljajićevo (S4-S6), and Trešnjevac (S7-S9); vertisol (4-6): Bečej (V1-V3), Mužlja (V4-V6), and Vršac (V7-V9); chernozem (7-8): Rimski Šančevi 1 and 2 (C1-C5), and Futog (C7-C9).
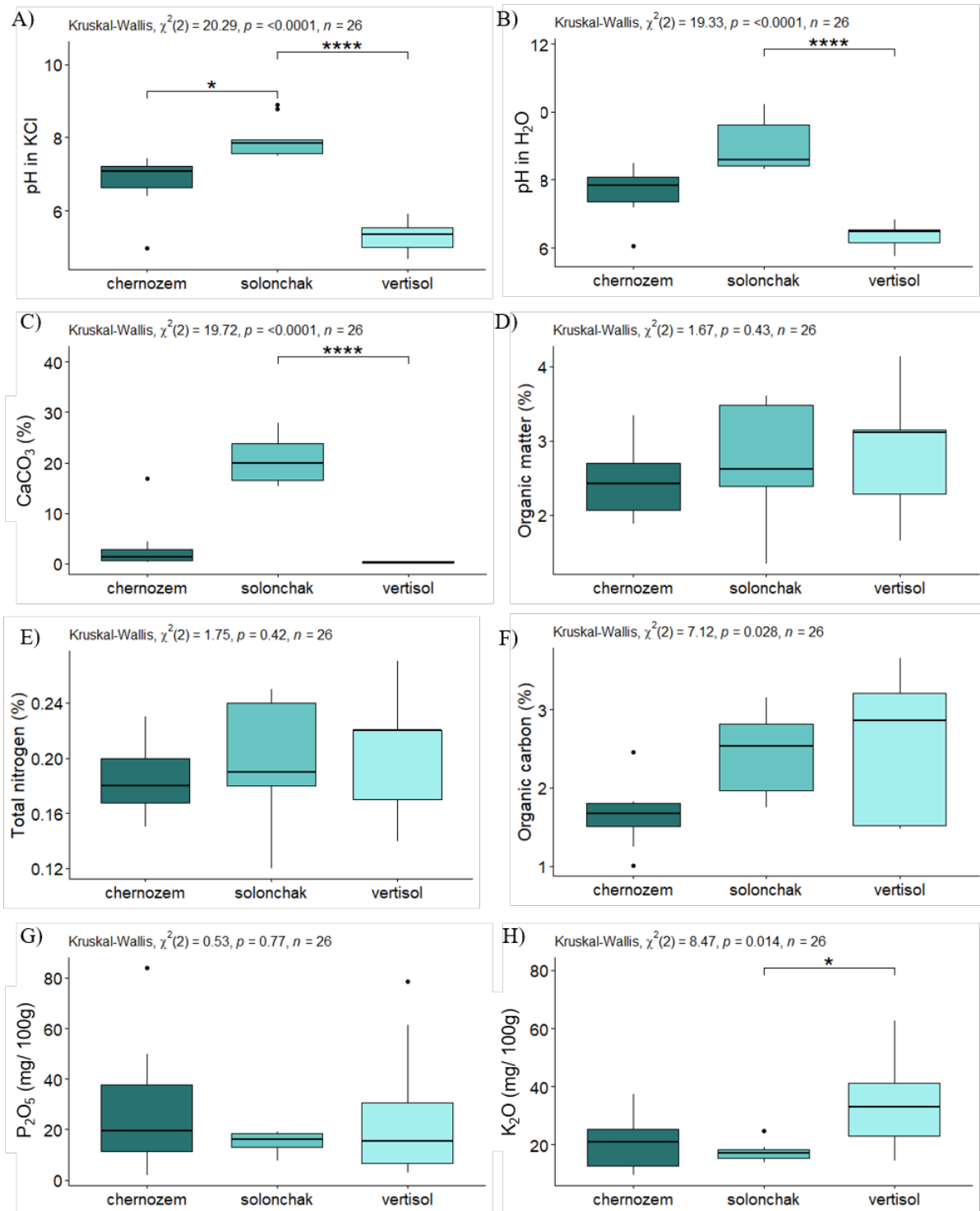
Supplementary Fig. S2. Differences in chemical properties across three different soil types. **A** – pH in KCl, **B** – pH in H_2O, **C** – $CaCO_3$ (%), **D** – organic matter (%), **E** – total nitrogen (%), **F** – organic carbon (%), **G** – P_2O_5 (mg/100 g), **H** – K_2O (mg/100 g).
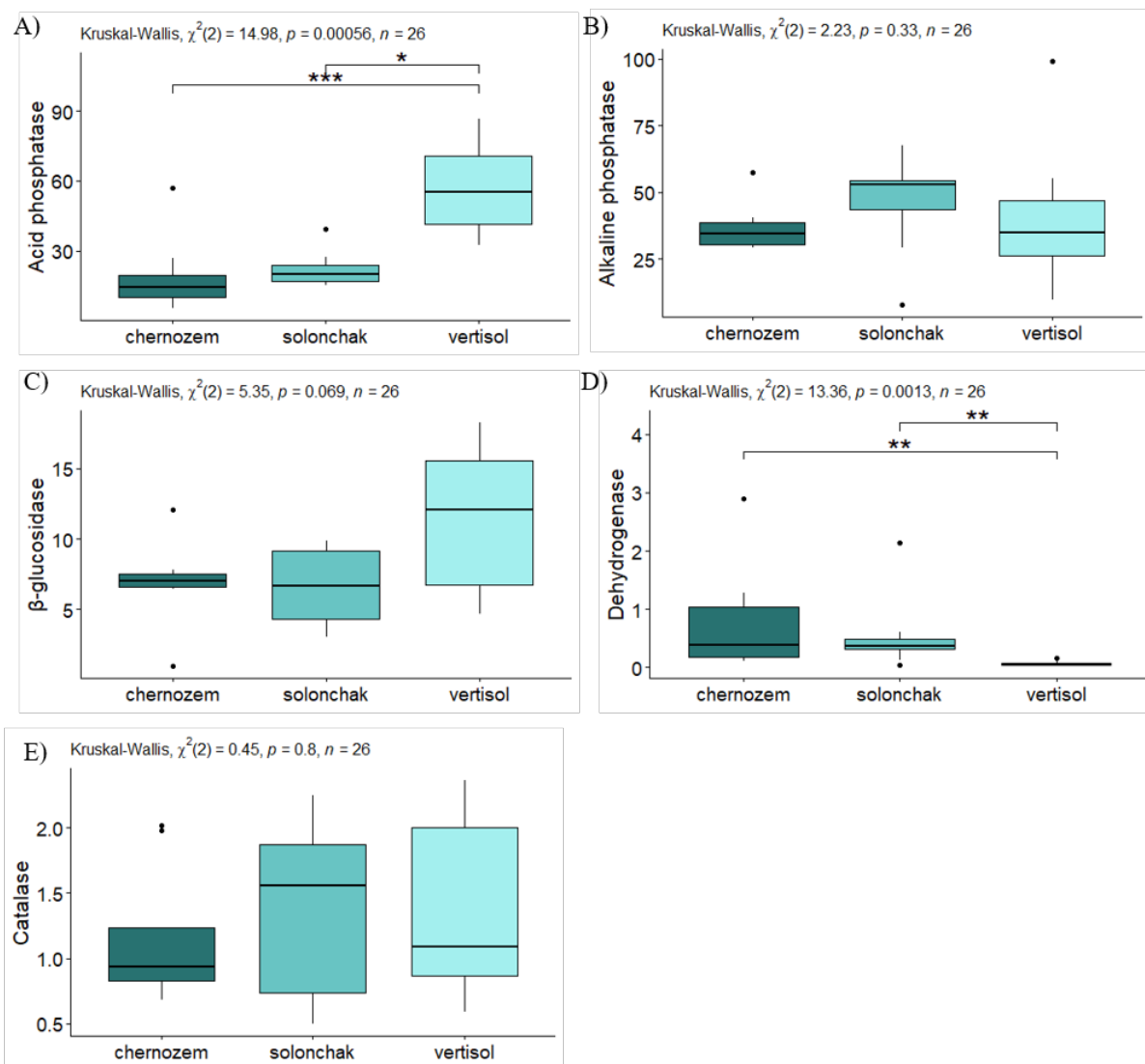
Supplementary Fig. S3. Differences in enzymatic activities across three different soil types. **A** – acid phosphatase (µg pNP g^{-1} dm h^{-1}), **B** – alkaline phosphatase (µg pNP g^{-1} dm h^{-1}), **C** – β-glucosidase (µg pNP g^{-1} dm h^{-1}), **D** – dehydrogenase (µg TPF g^{-1} dm h^{-1}), **E** – catalase (ml 0.2 M KMnO$_4$ g^{-1} dm h^{-1}).
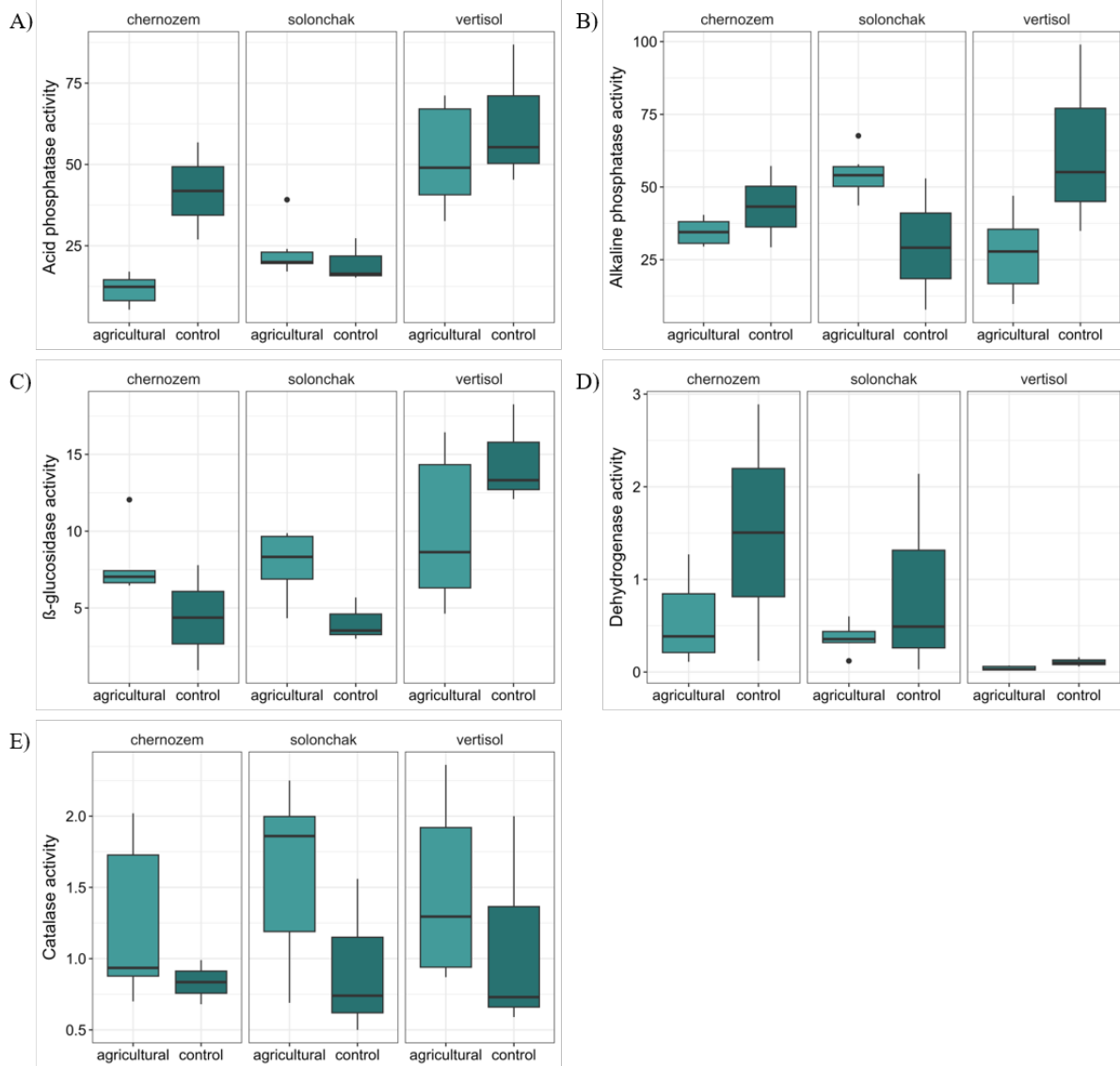
Supplementary Fig. S4. Enzymatic activity patterns across the type of land use (agricultural and control soil). **A** – acid phosphatase (μg pNP g^{-1} dm h^{-1}), **B** – alkaline phosphatase (μg pNP g^{-1} dm h^{-1}), **C** – β-glucosidase (μg pNP g^{-1} dm h^{-1}), **D** – dehydrogenase (μg TPF g^{-1} dm h^{-1}), **E** – catalase (ml 0.2 M KMnO$_4$ g^{-1} dm h^{-1}). Values are averaged by land use across all three soil types.