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Changes in soil physical properties under the influence of haypasture-colonizing successional grasses

Promjene u fizikalnim svojstvima tla nakon zarastanja livadnih pašnjaka sukcesijskim travama

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ABSTRACT

This study investigated the changes in soil physical properties after the abandonment of haypastures dominated by *Helictotrichon pubescens* and subsequent colonization by successional grasses *Brachypodium pinnatum* and *Calamagrostis epigejos*. We also investigated if there are linear relationships among the soil physical properties in the studied soils, and the results of the soil chemical property analysis were used as an aid in the interpretation of these relationships. The studied soil was calcocambisol on limestone. Soil samples were collected in disturbed and undisturbed state, and the differences among them were analysed by Mann-Whitney U test, whereas the relationships between soil properties were analysed with simple linear regression models. Soil solid particle density was higher in the B horizons of successional grasslands (median=2.61 g/cm^3) than in those of haypastures (median=2.54 g/cm^3). The A horizons of haypastures were barely 2–3 cm deep, whereas under successional grasses their depth reached as much as 25 cm. At the same soil depth (10–12 cm), the soil colonized by successional grasses had higher total porosity (median=53.3%) and lower bulk density (median=1.18 g/cm^3) than those of haypasture soil (medians of 45.1% and 1.41 g/cm^3, respectively). Soil total porosity and water holding capacity were positively linearly associated (r^2= 0.71, $P<0.0001$), but they were both negatively associated with bulk density (r^2=0.98 and r^2=0.67, respectively, $P<0.0001$), which was due to strong control of humus over the soil physical properties. We showed that the prevailing grassland type should not be ignored when studying physical properties of a specific soil type.

Keywords: vegetation succession, soil-vegetation relationships, calcocambisol, rotational grazing, land-use change

SAŽETAK

U ovom istraživanju analizirane su promjene fizikalnih svojstava tla nakon zapuštanja livadnih pašnjaka s dominacijom vrste *Helictotrichon pubescnens*, te posljedičnog zarastanja sukcesijskim travama *Brachypodium pinnatum* i *Calamagrostis epigejos*. Također, utvrđeno je postoje li linearni odnosi između fizikalnih svojstava tla, dok su rezultati kemijskih analiza korišteni za interpretaciju tih odnosa. Istraživanja su provedena na kalkokambisolu na vapnencu, a tlo je uzorkovano u narušenom i prirodnom stanju. Razlike između fizikalnih svojstava tla analizirane su pomoću Mann-Whitney U testa, a odnosi između fizikalnih svojstava tla analizirani su pomoću jednostavnih linearnih regresija. S obzirom na profile tla, B horizonti sukcesijskih travnjaka pokazali su veću gustoću čvrstih čestica (medijan=2.61 g/cm^3) od onih u livadnim pašnjacima (medijan=2.54 g/cm^3). A horizonti livadnih pašnjaka bili su jedva 2–3 cm duboki, dok se njihova dubina u sukcesijskim travnjacima produbila i do 25 cm. Iz praktične perspektive, na istoj dubini tla (10–12 cm), tla sukcesijskih travnjaka imala su veći ukupni porozitet (medijan=53,3%) od onoga u tlima livadnih pašnjaka (medijan=45.1%) te manju volumnu gustoću (medijan=1.18 g/cm^3) od one u tlima livadnih pašnjaka (medijan=1.41 g/cm^3). Ukupni porozitet i retencijski kapacitet tla za vodu pokazali su pozitivnu međusobnu linearnu povezanost (r^2= 0.71, P<0.0001), no oba svojstva su bila negativno linearno povezana s volumnom gustoćom tla (r^2=0.98 and r^2=0.67, respectively, P<0.0001), što je bila posljedica jakog utjecaja humusa na fizikalna svojstva tla. Ovo istraživanje pokazalo je da prevladavajući tip travnjaka treba uzeti u obzir kod istraživanja fizikalnih svojstava pojedinog tipa tla.

Ključne riječi: sukcesija vegetacije, odnosi tla i vegetacije, kalkokambisol, rotacijska ispaša, promjena načina korištenja zemljišta

INTRODUCTION

Some studies have shown that after the abandonment of pastures and meadows, different changes in soil physical properties occur along with the progression of vegetation succession, and these changes are often associated with various changes in soil chemical properties. It is well known that the presence of different plant species or vegetation types can induce various soil changes, and it has been shown that most plant-induced changes in soil physical properties occur through modifications of soil structure, which can be a consequence of mucilage exudation (Morel et al., 1991), nutrient release and exudation of C compounds (Pojasok and Kay, 1990), mycorrhizal associations (Wright and Upadhyaya, 1998; Auge et al., 2001; Wilson et al., 2009), or simply through organic matter input (see Angers and Caron, 1998). However, the overall number of studies that investigated such soil changes is still generally low, because soil scientists are rarely concerned about influence of different vegetation types on soil properties, whereas botanists and vegetation ecologists rarely study soil-vegetation relationships from the soil science perspective. Li and Shao (2006) showed that during vegetation succession on soils developed on loess, soil under the grass species *Stipa bungeana* Trin. showed the highest stability of soil structural aggregates among the soils in different successional stages. Moreover, they showed that the soil in this grass-dominated successional stage had a higher bulk density than that of the soil in scrub and forest stages. Pintaldi et al. (2016) showed that in sub-alpine regions of NW Italian Alps, colonization by grass species *Nardus stricta* L. causes the formation of soil hummocks (mounds) which show higher degree of podzolization than that of the adjacent soil in interhummocks. Compared to this adjacent soil which was dominated by dicotyledonous plant species, the soil under these hummocks had a lower nutrient content and soil pH, but a higher C content. Pei et al. (2008) found that grazing exclusion in an Inner Mongolian desert steppe led to a reduction in soil pH and soil bulk density and an increase in soil organic matter. Li et al. (2016) showed that grazing exclusion in alpine meadows of the Tibetan Plateau also led to the reduction in soil bulk density accompanied with the increase in soil water holding capacity; these changes were most apparent at 0–10 cm soil depth. Ren et al. (2018) also confirmed this in semi-arid grasslands of the China Loess Plateau; their study showed that the abandonment of pastures led to a decrease in soil pH and bulk density and an increase in soil capillary porosity, and thus water holding capacity, whereas it also led to the increase in soil organic matter content. The soil changes observed in their study were also most obvious in the upper soil horizon. However, few studies on soil changes after meadow and pasture abandonment (and consequent progression of vegetation succession) have assessed the changes in soil physical properties, as most of them only focused on changes in soil chemistry.

In Croatia, the abandonment of extensive livestock production in the last few decades has led to the significant loss of meadow and pasture habitats. Even though these grasslands types existed for centuries, maybe even millennia, their loss already occurs two to three years after their abandonment due to the progression of vegetation succession. However, before the development of scrub vegetation, most of these meadows and pastures are colonized by various successional grass species. In

Croatia, some floristic research regarding the succession of some grasslands have been carried out (Krstonošić et al., 2016), but no studies have been conducted on the soil changes that occur during this process.

In the present study, we investigated: (i) whether the abandonment of haypastures dominated by *Helictotrichon pubescens* (Huds.) Schult. & Schult. f. and subsequent colonization of these areas by successional grass species *Brachypodium pinnatum* (L.) P. Beauv. and *Calamagrostis epigejos* (L.) Roth leads to changes in the physical properties of calcocambisol on limestone in the karst region of NW Croatia, and (ii) whether physical properties of these soils are linearly related regardless of the dominating grassland type. The measured soil properties included soil bulk (volumetric) density (ϱb), soil water retention capacity (Cw), soil total porosity (P), soil solid particle density (ϱp), soil air capacity (Ca), soil pH, stability of soil structural aggregates, soil humus, and soil organic carbon content. The two mentioned successional grass species often form the first successional stage that occurs in our study area after the abandonment of the aforementioned haypastures. We refer to *H. pubescens*-dominated grasslands as „haypastures", which is a term also used by ter Braak (1987), because they are used as both pastures for low intensity rotational grazing by sheep and for obtaining hay as animal feed during the summer. Today, almost all grasslands in the study area are used as haypastures, and they are rarely used separately as pastures or meadows. In the study area, *B. pinnatum* sometimes forms homogenous successional grasslands without the accompanying species *C. epigejos*, but these stages were not analyzed in the present study; rather, soil properties were investigated under large, dense, and intertwined *B. pinnatum* and *C. epigejos* patches with highly developed aboveground biomass.

MATERIALS AND METHODS

Study site

The study site is located near the village Brlog Ozaljski, NW Croatia (45°37'32.58'', 14°24'12.16''). Mean annual rainfall in the study area is 1000–1100 mm and the mean annual temperature is 9–10°C (Zaninović et al., 2008). The study site is situated on a karst formation that mainly consist of biolithitic and bioclastic limestones (Bukovac et al., 1984). The dominant soil types that developed from these limestones in the study area are slightly lesivated (leached) terra rossas and calcocambisols, and the limestone rocks are often visible protruding above the soil surface. The dominant soil type at the study site is calcocambisol on limestone (see Husnjak, 2014). According to WRB classification, this soil type corresponds to cambisol group (WRB 2006). Even though *B. pinnatum* also forms homogenous successional grasslands after land abandonment in the study site, *C. epigejos* can be considered as its own "sinusia", as it rapidly clones itself and colonizes both haypastures and *B. pinnatum* grasslands without any specific pattern. However, its dominance is somewhat reduced within haypastures, probably because of the regular mowing during summer, but it peaks in abundance after the exclusion of mowing and grazing, resulting in intertwining with *B. pinnatum* in many locations.

Field study

Soil sampling was carried out on June 1st 2020, just a few days before the first summer mowing of the haypastures. Six soil profiles were dug up in three paired locations where the haypastures and successional grasslands grow in direct proximity to each other. On every location, one soil profile was dug up from each grassland type in a way that the dug up soil profiles of these two grassland types were positioned approximately ten meters apart. Within the successional grasslands, profiles were dug up under the dense and intertwined patches of *B. pinnatum* and *C. epigejos* that showed the highest aboveground biomass. From every soil profile, one disturbed soil sample from the A and one from the B horizon was collected. These samples were further analyzed for soil pH, humus content, stability of soil structural macroaggregates, and soil texture. For the analyses of Cw, ϱb ϱp, P, and Ca, we collected three undisturbed soil samples from A and B horizons of every soil profile under the successional grasslands, and only from B horizons under the haypastures, using Kopecky 100 cm^3 volume soil cores. In other words, undisturbed soil samples were collected in triplicate per soil profile. Core samples from the A horizons of haypastures were not collected, as the depth of the barely developed A horizon in this soil was only 2–3 cm, whereas the height of the cylinders was 5 cm. If these samples were collected, this would likely bias the pedological interpretation of our results. In total, there were 27 undisturbed soil core samples (9 from haypasture B horizons, 9 from successional grassland B horizons, and 9 from successional grassland A horizons) used for the analyses of soil physical properties.

Laboratory methods

Soil samples were analyzed at the Soil science department laboratory, Faculty of Agriculture, Zagreb where the basic soil properties were determined using the standard laboratory analyzes. All disturbed soil samples were air-dried. A portion of each sample was crushed and sieved through a 2 mm sieve. Soil particle size distribution was analyzed from disturbed soil samples by pipette-method, with wet sieving and sedimentation after dispersion with sodium-pyrophosphate ($Na_4P_2O_7$, c = 0.4 M, according to HRN ISO 11272:2004). Soil textural classes were interpreted according to FAO (2006). The stability of macroaggregates was determined according to the method of Sekere (JDPZ 1971). Soil pH was determined electrometrically in 1:2.5 H_2O and KCl suspension (HR ISO 10390:2005). Humus content was determined according to Tjurin method, by acid-dicromate ($K_2Cr_2O_7$, c=0.4 M) digestion (JDPZ 1966), and organic C content was calculated from the humus content according to Škorić (1982). The rest of the soil physical properties were analyzed in undisturbed samples. Soil water retention capacity (Cw), soil total porosity (P), and soil air capacity (Ca) were analyzed according to the method of Gračanin (Škorić 1982), whereas soil bulk density (ϱb) was analyzed according to HRN ISO 11272:2004, and the particle density (ϱp) was analyzed according to HRN ISO 11508:2004.

Statistical analyses

Statistical differences in soil physical properties between haypastures and successional grasslands were analyzed by Mann-Whitney U test for the equality of medians (H0: the two samples are taken from populations with equal medians) with a 95% confidence interval, as the normal probability plots showed that soil properties' data residuals were mostly non-normally distributed. Differences in the soil properties of the disturbed soil samples were not analyzed statistically because of the low number of measurements; rather, values of soil properties from the disturbed soil samples were used for the construction of scatterplots. These scatterplots served as an aid in the interpretation of the results of soil physical properties obtained from the undisturbed soil samples, and they were constructed using the PC-ORD 7.08 software (McCune and Mefford 2016). As the laboratory analyses of the undisturbed soil samples from haypastures were conducted using only the soil samples taken from B horizons, differences regarding the soil profiles of the two grassland types were thus analyzed only between the B horizons of the two grassland types. However, as the undisturbed soil samples from the A horizons of successional grasslands were obtained from the approximately same depth (10–12 cm) as those from the B horizons of haypastures, we also analyzed the differences in the soil physical properties between the successional grasslands' A horizons and haypastures' B horizons, as this would indicate the practical differences close to the soil surface before and after haypasture abandonment, regardless of the soil profiles of these soils. Common trends in the physical properties of soils from both grassland types were analyzed with simple linear regression models, as normal probability plots showed that residuals were quite normally distributed due to large sample size. All analyses were conducted using PAST 4.03 software (Hammer et al., 2001).

RESULTS AND DISCUSSION

Differences in the soil physical and basic chemical properties between haypastures and successional grasslands

The results of the laboratory analyses of soil physical and chemical properties of the disturbed soil samples are summarized in Table 1.

When considering the soil textural classes (FAO, 2006) of the studied calcocambisols and their soil horizons (Figure 1), soil samples from most locations were classified as a clay loam. The A horizon of successional grassland soil from location 2 was classified as loam, whereas in location 3, A and B horizon of haypasture were classified as clay, and B horizon of successional grassland was classified as the heavy clay.

The analyzed soil samples showed generally higher pH values in the haypastures than in the successional grasslands, with the greatest difference occurring in

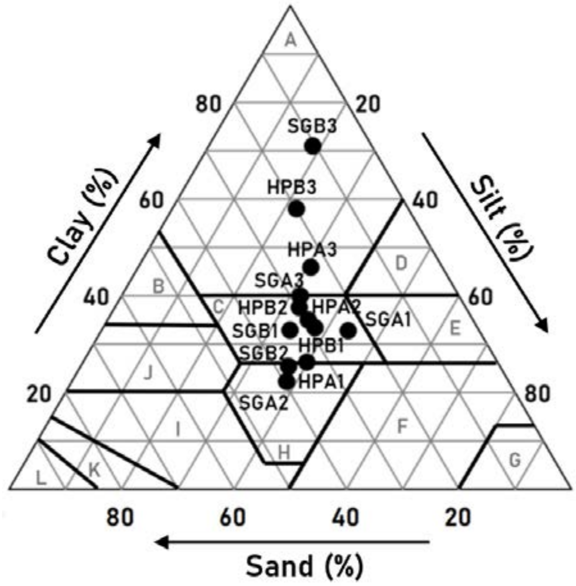

Figure 1. Textural triangle showing the textural classes of the studied soils, based on the data from Table 1. Abbreviations: HPA and HPB – haypastures' A and B horizons, respectively; SGA and SGB – successional grasslands A and B horizons, respectively; 1,2,3 – paired location number; A – clay, B – sandy clay, C – clay loam, D – silty clay, E – silty clay loam, F – silt loam, G – silt, H – loam, I – sandy loam, J – sandy clay loam, K – loamy sand, L - sand

Table 1. Chemical and physical properties of disturbed soil samples

	Horizon	*A* (n=3)			*B* (n=3)		
Grassland type		*min*	*max*	*median*	*min*	*max*	*median*
Haypastures (HP) *Soil type: Calcocambisol*	pH H_2O	5.56	6.53	5.72	6.15	6.50	6.28
	pH KCl	4.75	6.07	4.80	4.97	5.78	5.30
	Humus (%)	10.7	15.4	12.4	5.02	7.55	6.21
	Organic C (%)	6.21	8.94	7.20	2.91	4.38	3.60
	Sand 2.0–0.063 mm (%)	23.4	33.9	29.3	19.9	29.6	28.9
	Silt 0.063–0.002 mm (%)	30.7	39.8	35.7	22.1	37.7	32.8
	Clay <0.002 mm (%)	26.3	45.9	35.0	33.4	58.0	37.6
Successional grasslands (SG) *Soil type: Calcocambisol*	pH H_2O	5.30	5.61	5.56	5.39	6.24	5.55
	pH KCl	4.26	4.62	4.42	3.95	4.56	4.06
	Humus (%)	6.72	12.6	11.4	1.55	4.40	2.59
	Organic C (%)	3.90	7.32	6.63	0.90	2.55	1.50
	Sand 2.0–0.063 mm (%)	23.3	39.5	28.2	10.5	37.6	33.6
	Silt 0.063–0.002 mm (%)	31.8	43.9	38.2	18.5	37.0	33.5
	Clay <0.002 mm (%)	22.3	40.0	32.8	25.4	71.0	32.9

the paired location 3, in both A and B horizons, with a decrease of well over one unit on the pH scale in successional grasslands compared to that in haypastures. This indicated that the successional grasslands were a more acidic medium than the haypastures (Figure 2a). The same trend in the reduction of soil pH was found by Catorci et al. (2011) after the colonization of sub-Mediterranean meadows by *Brachypodium genuense* (DC.) Roem. & Schult. The pH measured in KCl in our study was expectedly 0.5 to 1.0 units lower than that measured in H_2O due to more exchangeable H^+ ions brought into the solution by using salt (KCl) (McCauley et al., 2009).

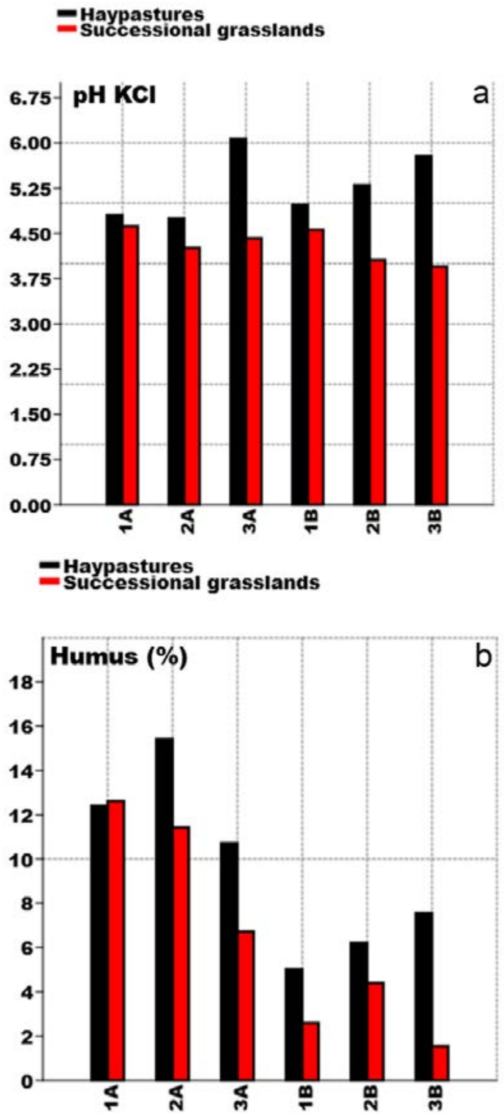

Figure 2. Soil pH (a) and humus content (b) in A and B horizons of haypastures and successional grasslands, based on the data from Table 1. Abbreviations: numbers on the horizontal axis represent the numbers of paired locations from which the soil was analyzed; A – A horizons, B – B horizons

Humus content was generally higher in haypastures than in successional grasslands except for location 1 (horizon A), where its content was almost equal in both grassland types. As shown in Figure 2b, humus values were generally higher in A horizons than in B horizons. Higher humus and soil organic C content in the haypastures than in successional grasslands were expected in this type of habitat. Even though rotational grazing by sheep in these haypastures is of low intensity, the manure produced by sheep increases the soil humus and organic C content in haypasture soil. In addition, leaching of the humus substances by rain is likely responsible for the high humus content in haypastures' B horizons. However, besides the high humus content in haypastures, the organo-mineral A horizons of haypastures were barely 2–3 cm deep, whereas after their abandonment and subsequent development of successional grasses, A horizons reached the depth of up to 25 cm (Table 2).

It is important to emphasize that most soil samples showed great aggregate stability (Table 2), which was related to high organic matter and organic C content as shown by Benito and Diaz-Fierros (1992).

When considering the changes in soil physical properties in relation to soil profiles, a significant change in ϱp was recorded in the B horizons after the colonization by successional grasses; ϱp was somewhat higher in the successional grasslands than in the haypastures as indicated by the Mann-Whitney U test ($P = 0.006$) (Table 3). This was a consequence of the lower humus content in B horizons of the successional grasslands than that in B horizons of the haypastures, as ϱp was positively associated with humus content (Figure S1m, supplementary data). Soil ϱb, P, and Cw values of B horizons did not change significantly after haypasture colonization by successional grasses.

However, when considering the changes in soil physical properties before and after the abandonment of haypastures near the soil surface, from a practical point of view, (i.e., differences occurring at soil depth of 10–12 cm regardless of the soil profiles), the abandonment of rotational grazing of haypastures and subsequent development of successional grasses led to the decrease in soil ϱb (P = 0.004) and increase in soil P (P = 0.001), as indicated by the Mann-Whitney U test (Table 4). The decrease in ϱb may have partly been a consequence of the exclusion of trampling by sheep, but it was predominantly a consequence of the higher humus content in successional grasslands than that in haypastures at this depth, and probably because of the very deep and dense

Table 2. Depth and aggregate stability of the analyzed soil horizons

Grassland type		Depth		Aggregate stability	
	Location	A horizon	B horizon	A horizon	B horizon
Haypastures (HP)	1	0–2 cm	>2 cm	Completely stable	Quite stable
	2	0–2 cm	>2 cm	Completely stable	Completely stable
	3	0–3 cm	>3 cm	Completely stable	Quite stable
Successional grasslands (SG)	1	0–25 cm	>25 cm	Completely stable	Somewhat stable
	2	0–16 cm	>16 cm	Completely stable	Quite stable
	3	0–23 cm	>23 cm	Completely stable	Completely unstable

Table 3. Differences in soil physical properties between the B horizons of haypastures (during low intensity rotational grazing) and those of successional grasslands (after the abandonment of rotational grazing), according to the Mann-Whitney U test (n=9)

	Haypastures			*Successional grasslands*				
	min	*max*	*median*	*min*	*max*	*median*	P	U
ϱb (g/cm³)	1.29	1.47	1.41	1.22	1.56	1.50	0.250	27.0
ϱp (g/cm³)	2.50	2.62	2.54	2.58	2.64	2.61	**0.006**	9.50
P (%)	42.3	49.4	45.1	40.2	53.7	42.7	0.377	30.0
Cw (%)	32.6	43.1	39.0	33.6	48.4	35.2	0.536	33.0
Ca (%)	3.42	12.5	6.46	3.63	8.52	6.75	0.930	39.0

Abbreviations: ϱb – soil bulk density, ϱp – soil solid particles density, P (%) – soil total porosity, Cw (%) – soil water retention capacity, Ca (%) – soil air capacity; values in bold represent statistically significant differences between the medians (P<0.05)

Table 4. Differences in soil physical properties between the soils of haypastures (during low intensity rotational grazing) and those of successional grasslands (after the abandonment of rotational grazing) measured at the same soil depth (10–12 cm), according to the Mann-Whitney U test (n=9)

	Haypastures			*Successional grasslands*				
	min	*max*	*median*	*min*	*max*	*median*	P	U
ϱb (g/cm³)	1.29	1.47	1.41	1.08	1.33	1.18	**0.004**	7.50
ϱp (g/cm³)	2.50	2.62	2.54	2.46	2.58	2.53	0.095	21.5
P (%)	42.3	49.4	45.1	48.4	57.0	53.3	**0.001**	4.00
Cw (%)	32.6	43.1	39.0	37.7	50.3	40.0	0.133	23.0
Ca (%)	3.42	12.5	6.46	5.49	13.9	9.81	0.093	21.0

Abbreviations: ϱb – soil bulk density, ϱp – soil solid particles density, P (%) – soil total porosity, Cw (%) – soil water retention capacity, Ca (%) – soil air capacity; values in bold represent statistically significant differences between the medians (P<0.05) root system of *B. pinnatum* and *C. epigejos*, which were likely to mitigate soil compaction. The increase in P at this depth in successional grasslands was thus mostly a consequence of humus-induced decrease in ϱb (see Figure S1d and S1h, supplementary data). Organic matter is generally known to increase the number of soil pores of all sizes, and it acts as their stabilizer while also increasing their persistence when exposed to stress (Kay and Van den Bygaard, 2002). Even though Cw, Ca, and ϱp varied across the studied paired locations, the abandonment of rotational grazing and development of successional grasses did not have a statistically significant influence on the values of these soil physical properties at the same soil depth according to the Mann-Whitney U test (Table 4).

Linear relationships between the soil physical properties in the studied grasslands' soils

Certain interesting relationships between some of the soil physical properties were observed in the analyzed calcocambisol on limestone regardless of dominating grassland type (Figure 3). P showed a fully linear decrease with the increase in ϱb (Figure 3a). The increase in Cw could be predicted by increase in P (Figure 3c), thus, Cw also showed a linear decrease with the increase in ϱb (Figure 3b). However, the residuals in the regression model of Cw and P, and Cw and ϱb were more unevenly distributed compared to those in the regression model of P with ϱb. Soil Ca could not be predicted by any of the soil physical properties, thus, the regression models were not displayed for Ca.

The decrease in ϱb led to the increase in Cw and P, which was a consequence of humus presence, as the increase in humus content was mostly responsible for the decrease in ϱb (Figure S1d, supplementary data). Due to positive association between Cw and P, we could assume that P likely also somewhat accounted for the water holding pores. In addition, Ca could not be predicted by P, Cw, and ϱb; thus, the mentioned increase in P was a consequence of increasing humus and clay content (Figure S1e and S1h, supplementary data), which could have also led to the increase in soil micro and medium

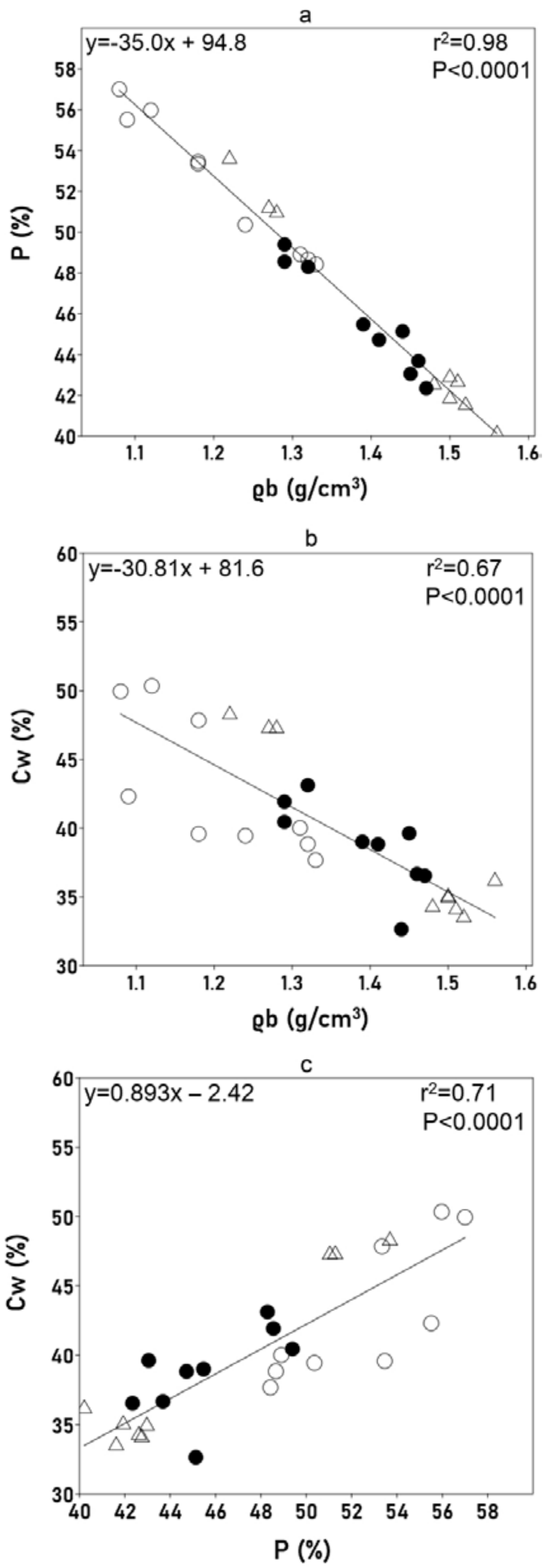

Figure 3. Simple linear regression models between the soil physical properties (soil bulk density, soil water holding capacity, and soil total porosity). Abbreviations: ϱb – soil bulk density, P (%) – soil total porosity, Cw (%) – soil water retention capacity; filled dots – haypastures' B horizons, triangles – successional grasslands' B horizons, empty dots –successional grasslands' A horizons

pores content, as shown by Kirchmann and Gerzabek (1999). Both humus and clay content are known from before to have a strongest control over the various soil physical properties in pseudogley (Rubinić and Husnjak, 2016). However, the reason why Ca and Cw were not significantly negatively linearly associated in these soils was probably because Cw was not a consequence of water holding pores only, rather it was also influenced by various organic compounds originating from humus, which have high water absorption efficiency.

In addition, in the studied calcocambisols, high stability of soil structural aggregates as well as the presence of humus, seemed to have a great influence on the soil physical properties, and as a consequence, the clay fraction was not responsible for the increase in soil ϱb (Figure S1a, supplementary data). We assumed that this was due to the formation of strong organo-mineral complexes between the clay and organic matter, which consequently resulted in high stability of soil structural aggregates and low influence of clay fraction on ϱb (see Chenu et al., 1998). Because of this, silt and sand content seemed to be somewhat positively associated with soil ϱb (Figure S1b and Figure S1c, respectively, supplementary data). However, this occurred indirectly because these fractions mostly do not bind with humus substances to form organo-mineral complexes, as is the case with clay fractions. In addition, sand and silt hardly form highly stable aggregates, which is why in this case, they remained in a compact state, compared to clay which was mostly bound to organo-mineral complexes which may have had a lower density than pure clay, sand, or silt fractions, and which are resistant to biodegradation (Chenu and Plante, 2006). We can therefore conclude that in the studied calcocambisols, regardless of the grassland type, humus was a feature that had the strongest control over the soil physical properties (Figure S1d, S1h, S1l, S1m, supplementary data), even though it originated from different sources in the two grassland types (i.e., humus in the haypastures mostly originated from sheep manure, whereas humus in the successional grasslands originated from the slowly decomposing leaves and dead roots of *B. pinnatum* and *C. epigejos*).

Considering the data from our study and the data from previous studies on similar topics, we can conclude that the influence of vegetation on soil should not be ignored, as it is a significant factor to consider when studying the properties of a specific soil type. Even within grassland vegetation, the prevailing grassland type should be taken into consideration in soil science research. We recommend that the soil changes occurring due to prevalence of various grassland types and due to land use change should be studied on different soil types, in order to distinguish the influence of vegetation from other soil forming factors.

CONCLUSION

In the present study, we found that after the abandonment of haypastures dominated by *Helictotrichon pubescens* on calcocambisol on limestone, and subsequent colonization by successional grasses *Brachypodium pinnatum* and *Calamagrostis epigejos*, there was a decrease in soil bulk density and soil total porosity near the soil surface, and increase in particle density in soil B horizons. Humus content and pH of soil A and B horizons generally decreased after the development of successional grasses. The depth of A horizons in haypastures was barely 2–3 cm, whereas under successional grasses, this depth even reached 25 cm. We also showed that humus, regardless of its origin, was a soil chemical property that exhibited the highest influence on soil physical properties in the studied calcocambisol. Further studies in the fields of soil science and vegetation ecology should focus on soil-vegetation relationships.

AUTHOR CONTRIBUTIONS

Study conceptualization and manuscript preparation: A. J., K. Ž., and D. S.; field soil sampling: I. M. and A. J.; soil laboratory analyses: K. Ž. and I. M.; calculations of soil properties from the laboratory data: D. S.; statistical analyses: A. J. and D. S.; mentorship during the project: M. S. The study originated from a professional project conducted by A. J., K. Ž., and D. S. during their master's studies in agroecology.

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REFERENCES

Angers, D. A., Caron, J. (1998) Plant-induced changes in soil structure: Processes and feedbacks. Biogeochemistry, 42 (1), 55-72. DOI: https://doi.org/10.1007/978-94-017-2691-7_3

Augé, R. M., Stodola, A. J. W. Tims, J., Saxton, A. (2001) Moisture retention properties of a mycorrhizal soil. Plant and Soil, 230 (1), 87–97. DOI: https://doi.org/10.1023/A:1004891210871

Benito, E., Diaz-Fierros, F. (1992) Effects of cropping on the structural stability of soils rich in organic matter. Soil & Tillage Research, 23 (1-2), 153-161. DOI: https://doi.org/10.1016/0167-1987(92)90011-Y

Bukovac, J., Poljak, M., Šušnjar, M., Čakalo, M. (1983) Osnovna geološka karta SFRJ 1:100 000. List Črnomelj, L 33-91. Geološki zavod Zagreb i Geološki zavod Ljubljana. Izdavač: Savezni geološki zavod, Beograd.

Catorci, A., Cesaretti, S., Gatti, R., Ottaviani G. (2011) Abiotic and biotic changes due to spread of *Brachypodium genuense* (DC.) Roem. & Schult. in sub-Mediterranean meadows. Community Ecology, 12 (1), 117–125. DOI: https://doi.org/10.1556/ComEc.12.2011.1.14

Chenu, C., Plante, A. (2006) Clay-sized organo-mineral complexes in a cultivation chronosequence: Revisiting the concept of the 'primary organo-mineral complex'. European Journal of Soil Science, 57. 596 - 607. DOI: https://doi.org/10.1111/j.1365-2389.2006.00834.x

Chenu, C., Puget, P., Balesdent, J. (1998) Clay–organic matter associations in soils: microstructure and contribution to soil physical stability. In: 16th World Congress of Soil Science Proceedings, (ed. A. Ruellan). Cirad, Montpellier, France

FAO (2006) Guidelines for soil description – fourth edition. Publishing management service, Rome.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001). Past: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4 (1), 9.

HRN ISO (10390:2005) Soil quality – Determination of pH. Zagreb: Croatian Standards Institute.

HRN ISO (11272:2004) Soil quality – Determination of dry bulk density. Zagreb: Croatian Standards Institute.

HRN ISO (11464:2009) Soil quality – Pretreatment of samples for physico-chemical analysis. Zagreb: Croatian Standards Institute.

HRN ISO (11508:2004) Soil quality – Determination of particle density. Zagreb: Croatian Standards Institute.

Husnjak, S. (2014) Sistematika tala Hrvatske. Hrvatska sveučilišna naklada, Zagreb. ISBN: 9789531692670

IUSS Working group WRB (2006) World reference base for soil resources 2006. World Soil Resources report No. 103, FAO, Rome.

JDPZ (1966) Priručnik za ispitivanje zemljišta. Knjiga I. Kemijske metode ispitivanja zemljišta. Beograd.

JDPZ (1971) Priručnik za ispitivanje zemljišta. Knjiga V. Metodika ispitivanja fizičkih svojstava zemljišta. Beograd.

Kay, B. D., VandenBygaard, A. J. (2002) Conservation tillage and depth stratification of porosity and soil organic matter. Soil & Tillage Research 66 (2), 107-118. DOI: https://doi.org/10.1016/S0167-1987(02)00019-3

Kirchmann, H., Gerzabek, M.H. (1999) Relationship between soil organic matter and micropores in a long-term experiment at Ultuna, Sweden. Journal of Plant Nutrition and Soil Science, 162 (5), 493–498. DOI: https://doi.org/10.1002/(SICI)1522-2624(199910)162:5<493::AID-JPLN493>3.0.CO;2-S

Krstonošić, D., Guzmić, M., Franjić, J., Škvorc, Ž., Sever K. (2016) Flora termofilnih travnjaka u sukcesiji na južnim obroncima Papuka. Glasnik Hrvatskog botaničkog društva 4 (1), 4-21

Li, H., Zhang, F., Mao, S., Zhu, J., Yang, Y., He, H., Li, Y. (2016) Effects of grazing exclusion on soil properties in Maqin alpine meadow, Tibetan Plateau, China. Polish Journal of Environmental Studies, 25 (4), 1583. DOI: https://doi.org/10.15244/pjoes/62099

Li, Y.Y., Shao, M.A. (2006) Change of soil physical properties under long-term natural vegetation restoration in the Loess Plateau of China. Journal of Arid Environments, 64 (1), 77-96. DOI: https://doi.org/10.1016/j.jaridenv.2005.04.005

McCauley, A., Jones, C., Jacobsen, J. (2009) Soil pH and organic matter. Nutrient management module 8 (2), 1–12.

McCune, B., Mefford, M. J. (2016) PC-ORD. Multivariate Analysis of Ecological Data, Version 7 . MjM Software Design, Gleneden Beach, Oregon, U.S.A.

Morel, J.L., Habib, L., Plantureux, S., Guckert, A. (1991) Influence of maize root mucilage on soil aggregate stability. Plant and Soil, 136 (1), 111-119. DOI: https://doi.org/10.1007/BF02465226

Pei, S., Fu, H., Wan, C. (2008) Changes in soil properties and vegetation following exclosure and grazing in degraded Alxa desert steppe of Inner Mongolia, China. Agriculture, Ecosystems & Environment, 124 (1-2), 33-39. DOI: https://doi.org/10.1016/j.agee.2007.08.008

Pintaldi, E., D'Amico, M.E., Siniscalco, C., Cremonese, E., Celi, L., Filippa, G., Prati, M., Freppaz, M. (2016) Hummocks affect soil properties and soil-vegetation relationships in a subalpine grassland (North-Western Italian Alps). Catena, 145: 214-226. DOI: https://doi.org/10.1016/j.catena.2016.06.014

Pojasok, T., Kay, B.D. (1990) Effect of root exudates from corn and bromegrass on soil structural stability. Canadian Journal of Soil Science, 70 (3), 351-362. DOI: https://doi.org/10.4141/cjss90-036

Ren, G., Wang, C., Dong, K., Zhu, H., Wang, Y., & Zhao, X. (2018) Effects of grazing exclusion on soil–vegetation relationships in a semiarid grassland on the Loess Plateau, China. Land Degradation & Development, 29 (11), 4071-4079. DOI: https://doi.org/10.1002/ldr.3164

Rubinić, V., Husnjak, S. (2016) Clay and humus contents have the key impact on physical properties of Croatian pseudogleys. Agriculturae Conspectus Scientificus, 81 (4), 187-191.

Škorić, A. (1982) Priručnik za pedološka istraživanja. Fakultet poljoprivrednih znanosti, Zagreb.

Ter Braak, C.J.F. (1987) The analysis of vegetation-environment relationships by canonical correspondence analysis. Vegetatio, 69 (1), 69-77. DOI: https://doi.org/10.1007/BF00038688

Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., Hartnett, D.C. (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecological Letters, 12 (5), 452–461. DOI: https://doi.org/10.1111/j.1461-0248.2009.01303.x

Wright, S. F., Upadhyaya, A. (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil, 198 (1), 97–107. DOI: https://doi.org/10.1023/A:1004347701584

Zaninović, K., Gajić-Čapka, M., Perčec, T, M., Vučetić M., Milković J., Bajić A., Cindrić K., Cvitan L., Katušin Z., Kaučić D., Likso T., Lončar E., Lončar Ž., Mihajlović D., Pandžić K., Patarčić M., Srnec L., Vučetić V., Likso, T. (2008) Klimatski atlas hrvatske. Državni hidrometeorološki zavod, Zagreb.

DOI: /10.5513/JCEA01/22.4.3267

SUPPLEMENTARY DATA

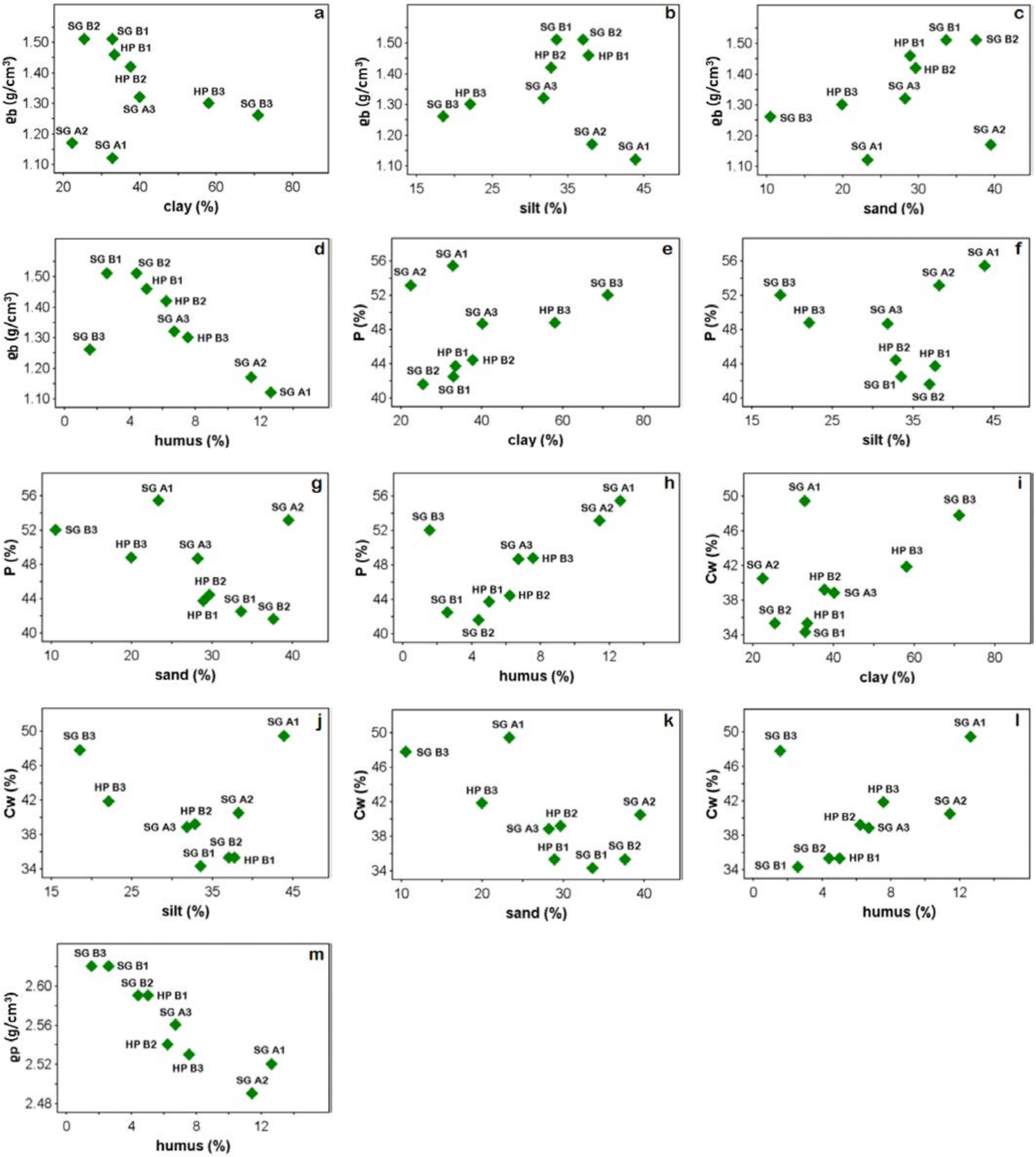

Figure S1. Scatterplots of soil properties from the studied soils

Abbreviations: ϱb – soil bulk density, ϱp – soil solid particle density, P (%) – soil total porosity, Cw (%) – soil water holding capacity; from every soil horizon analyzed, values of ϱb, ϱp, Cw, and P were averaged in order to be comparable with the soil properties obtained from the disturbed soil samples