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Soil organic carbon stock capacity in karst dolines under different land uses

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ABSTRACT

The soil organic carbon (SOC) was determined in soils of enclosed karst depressions (dolines) (NW Dinaric Mts.) to define their potential for organic carbon sequestration. SOC was measured in the forest, succession (scrubland), and grassland plots at the bottom of dolines at four depths (0–40 cm) and for 40 cm soil layer SOC stock was calculated. We demonstrated that the prevailing fine soil fractions, the C/N ratio and soil thickness play a positive role in the storage capacity of SOC in dolines regardless land use type. Grasslands have the lowest SOC storage capacity (106 t/ha/40 cm), while the highest SOC storage capacity is in succession plots (130 t/ha/40 cm). The last are covered by shrub communities dominated by *Prunus spinosa*, forming dense communities, and are typical of abandoned croplands or meadows that have been impacted by high levels of nutrients during cultivation phase. At this stage, there is no additional nutrient input in studied plots, which lowers the nutrient content and increases the C/N ratio. C/N ratio is the highest in the forest, where SOC stock capacity is 116 t/ha/40 cm. Given the trend towards the abandonment of agricultural land at Kras Plateau (SW Slovenia), we can expect more overgrowth of dolines, and thus an increase in carbon stocks and stabilization of organic carbon in forest soils. In contrary, we noticed the alarming decrease in grasslands and increase in urban land. The SOC storage in 2020 was for 12,538 t/ha/40 cm lower than in 2002. Although grasslands showed the lowest SOC storage, their contribution to total SOC storage in dolines is very important. Since there is a lack of studies on carbon stocks in doline soils, our research is of great importance and a novelty and gives an important background for further research on SOC stock in karst landscapes worldwide.

1. Introduction

Karst landscapes, which account for 20 % of dry ice-free land worldwide, are characterized by soluble and fractured carbonate bedrock (Ford and Williams, 2007) and are holding a significant potential for carbon sequestration (Gombert, 2002; Liu et al., 2013). The global volume of organic carbon (Corg) stored in karst aquifers was estimated at 200 km^3 (Veni, 2013). Many studies have calculated also the concentrations of organic carbon in top-soils in different karst landscapes (e.g., Liu et al., 2013; Yang et al., 2019). Li et al. (2015) considered land use changes and investigated soil organic carbon (SOC) and total Nitrogen (TN) in cropland, grassland, scrubland and secondary forest. They found that the contents of SOC and TN were significantly higher on limestone than on dolomite. However, the stock of SOC has been much less explored (Djuma et al., 2020) because sampling needs to be performed deeper in the soil profile by specific methodology. Measuring SOC concentrations (% or g/kg) in soil alone is not representative of carbon storage (t/ha) and could easily be misinterpreted (Eldon and Gerhenson, 2015; Djuma et al., 2020). Chen et al. (2012) found that the maximum SOC stock in the karst area is 3.5 times higher than that in non-karst area.

The spatial and temporal distribution of SOC depends on a number of mechanisms that include climatic variables (Davidson and Janssens, 2006; Turrión et al., 2009; Luo et al., 2017), the quantity and quality of soil organic matter (SOM), soil conditions, topography (Bergstrom et al., 2001; Román-Sánchez et al., 2018; Kobler, 2019; Patton et al., 2019), and parent material (Jobbágy and Jackson, 2000). Due to considerable heterogeneity associated with topography and soil (e.g. different thickness, water-retention, mineral composition) the distribution of SOC stocks in karst is difficult to estimate. In the Dinaric karst, limestone slopes are usually covered by shallow rendzinas (Rendzic Leptosol) and dolomite slopes by thicker Chromic Cambisol (Grčman et al., 2015; Zorn

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et al., 2020). The typical texture of these soils is silty-clay. Soils deeper than 0.5 m are relatively rare; they are associated with topographic depressions (e.g., dolines, called also sinkholes), laterally enlarged fractures, or old denuded caves filled with sediments (Yang et al., 2019) and contain a higher proportion of silt than clay in the upper 10 cm (Breg Valjavec et al., 2018a, 2018b).

Dolines are sediment traps and the sediment content covers the underlying rock formations (Sauro, 2009). In the last two decades, much attention has been paid to the study of dolines in view of the ongoing land degradation (Breg Valjavec et al., 2018a, 2018b) to identify the negative impacts on soil and vegetation and to stop these anthropogenic processes and start conservation measures. Many other studies supported the conservation value of dolines, especially from a biodiversity perspective, and highlighted many aspects of dolines (Bátori et al. 2009, 2014, 2019; Kiss et al. 2020; Aguilon et al. 2020), such as their function as refugia for endangered cold-adapted plant species.

In this study, we focus on the thick soil layer that is characteristic for solution dolines, and accumulated at their bottoms. The soils in the uppermost layer of 40 cm are investigated to obtain data on the vertical dynamics, stock and stability of SOC. We studied the role of dolines in soil organic carbon storage (SOC) in the mineral part of the soil as the soils are considered the largest terrestrial reservoir of organic carbon (Batjes, 1996; Cheng et al., 2015; Toensmeier, 2016). Studies of SOC stocks in dolines are completely lacking, so we want to evaluate the role of dolines as (potential) soil organic carbon pools. In some dolines, sediments can be up to 15 m thick (Habič, 1978), which theoretically allows for much greater accumulation of SOC than in the much shallower soils outside of dolines. The soils at the bottom of dolines are not characterised as colluvium soils only, but have a complex stratigraphy influenced by various transport processes (underground transport, wind, etc.) (Zupančič et al., 2018) as well as by recent and paleo-processes. Our previous studies of the top soil layer (10 cm mineral soil) in several semi-natural grassland dolines of Kras Plateau (SW Slovenia) (Breg Valjavec et al., 2018a, 2018b) revealed higher mean values of nutrients (0.49 % N, 0.0421 % S), higher Corg and higher Sulphur values, and lower reaction (pH = 6.27) compared to the soils of the surrounding plateau.

Additionally, we investigated and compared the SOC stocks in soils in dolines under three land use types: forests, grasslands, and succession (scrubland to towards natural afforestation) and tried to evaluate the role of current and past land use by analysing land use changes. Land use is an important factor controlling soil organic carbon storage because it affects the amount, location, and composition of litter input, decomposition, and organic matter stabilization processes (John et al., 2005). The carbon stock in soils is either in a static equilibrium or it can change due to changes in management practices, climate, or land use (Prietzel, 2016). Ostle et al. (2009) noted that land use changes that accelerate the biotic (decomposition) and abiotic (disturbance, erosion) carbon cycling can occur rapidly, within years, and are very difficult to reverse in the short term. Therefore, it is very important to recognise that it may take decades to centuries to recover to the original soil carbon stock after land use change disturbance (Guo and Gifford, 2002). Land use changes can therefore alter the total amount of soil organic carbon stored in a relatively stable manner, as well as soil environmental factors such as temperature, moisture, and the accessibility of energy sources for soil organisms (Ellert and Bettany, 1995). Therefore, identifying the plant-soil ecosystem that promotes carbon sequestration is very important (Wilson and Puri, 2001). It is very important to know how each of the land uses studied affects carbon storage, as our further policies must include strategies to prevent or reduce soil carbon loss due to land use change and consider the trade-offs between managing to enhance carbon sequestration and maintaining food and energy security (Ostle et al., 2009). Following previous studies in karst regions (Post and Kwon, 2000; Liu et al., 2013; Yang et al., 2019), we hypothesised that the amount of SOC is higher in dolines, where the current land use is forest, followed by succession and grassland dolines. In addition, we emphasise the contribution of over 14,000 dolines of Kras Plateau to the SOC balance.

2. Study area and methods

2.1. Kras Plateau

Kras Plateau (429 km^2) with an average altitude of 330 m a. s. l is located on the NW edge of the Dinaric Mountains, SW Slovenia (Fig. 1). The climate is characterised as sub-Mediterranean, with rainy, cool winters and long, dry summers, while precipitation is about 1400 mm, distributed almost evenly throughout the year, with a mean annual air temperature of 10–12 °C (Ogrin, 1995).

The bedrock is composed of Mesozoic limestone. The predominant soil types at Kras Plateau are rendzinas (40 %) and brown cambisoils (50 %). Leached soils (10 %) occur infrequently (Grčman et al., 2015) and are most common in enclosed karst depressions (dolines). Leached soils are a result of highly concentrated surface runoff into the dolines. As they are several small polypedones below the minimum size of a pedogeographical unit, they are not shown on the soil map (Fig. 1). At Dinaric Mountains plateaus, soil is a scarce resource and deeper soils are only found at the bottom of dolines (Mihevc et al., 2010). Therefore, dolines have been transformed throughout history to adapt them to the needs of agriculture (Breg Valjavec et al., 2018b). In the Kras Plateau, dolines were mostly used as pastures, meadows and croplands. Due to small plots, dolines are not profitable agricultural land for today's modern cultivation, thus they are largely abandoned for agriculture. Fig. 1 provides a general introduction to the sampling sites distribution, doline land use and generalised soil map of the area (Grčman et al., 2015). Sampling was carried out in 22 dolines within the cultural heritage site - UNESCO Škocjan Caves Regional Park. The landscape is a more or less flat terrain with many dolines. The doline bottoms are between 10 m^2 and 100 m^2 in size.

The zonal vegetation of the area is a forest dominated by *Ostrya carpinifolia* and *Quercus pubescens*. Due to the particular cooler and moisture microclimate and accumulation of soil, dolines also contain forests dominated by *Fagus sylvatica, Carpinus betulus, Fraxinus excelsior* and *Tilia cordata* (Poldini, 1989; Dakskobler, 2006). After large-scale deforestation, which occurred centuries ago (Gams, 2000), semi-natural karstic pastures (*Carex humilis-Centaurea rupestris* community) and karstic meadows (*Danthonia calycina-Scorzonera villosa* community) developed. Recently, natural afforestation of entire Kras Plateau region is in progress (Kaligarič and Ivajnšič, 2014; Breg Valjavec et al., 2018a, 2018b). In the dolines Central European mesic grasslands dominated by *Arrhenatherum elatius* prevail (Kaligarič, 1997; Kaligarič et al., 2006).

2.2. Soil sampling, laboratory and statistical analysis

Soil organic carbon (SOC) refers to the carbon component of SOM measured in mineral soils passing through a 2-mm sieve. Soil samples were collected from 22 dolines in spring and summer 2018. Doline landform can be divided into four topographic units according to Li et al. (2008). In this study, soil samples were collected from the centres of the dolines (bottom) (Fig. 2A, 2B) at a radius of 1 m in three directions: North, Southeast, and Southwest (Fig. 2C). Soil samples were collected using 6.7 cm diameter metal core at the following depth levels: 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm, according to ICP methodology (Cools and De Vos, 2016). Thus, three soil samples were collected from four different soil depths at each site (Fig. 2C). Samples from the same depth were aggregated to one soil sample. The herb layer and all organic soil layers (Ol, Of and Oh) were not included in the soil samples. The total number of aggregated soil samples was 88. The study was conducted in the upper 40 cm where organic matter is concentrated and where mineralization and immobilisation processes are most active. The soil sampling methodology followed the ICP Forest (Cools and De Vos, 2016).

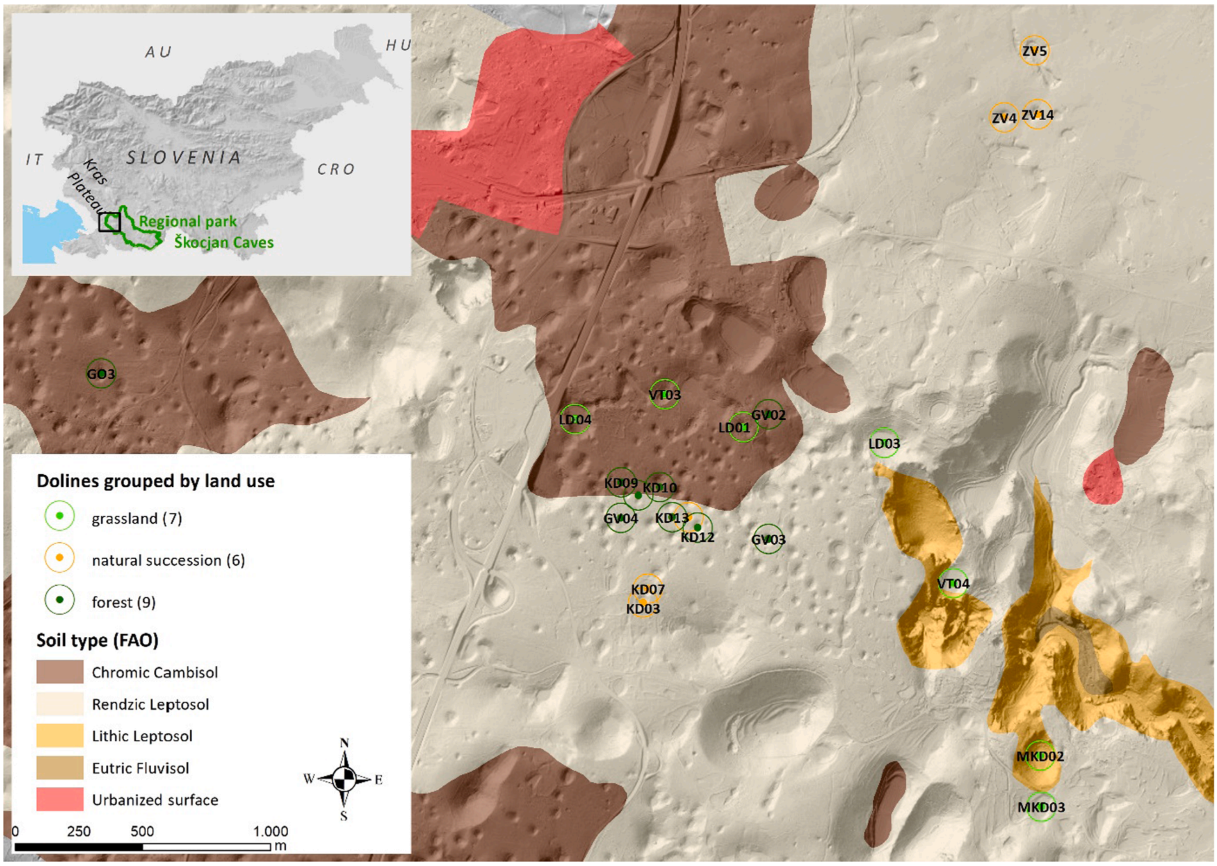

Fig. 1. Distribution of sampling sites (22) on Kras Plateau (Regional park Škocjan Cave) represented on shaded Lidar terrain and overlaid by generalised soil map.

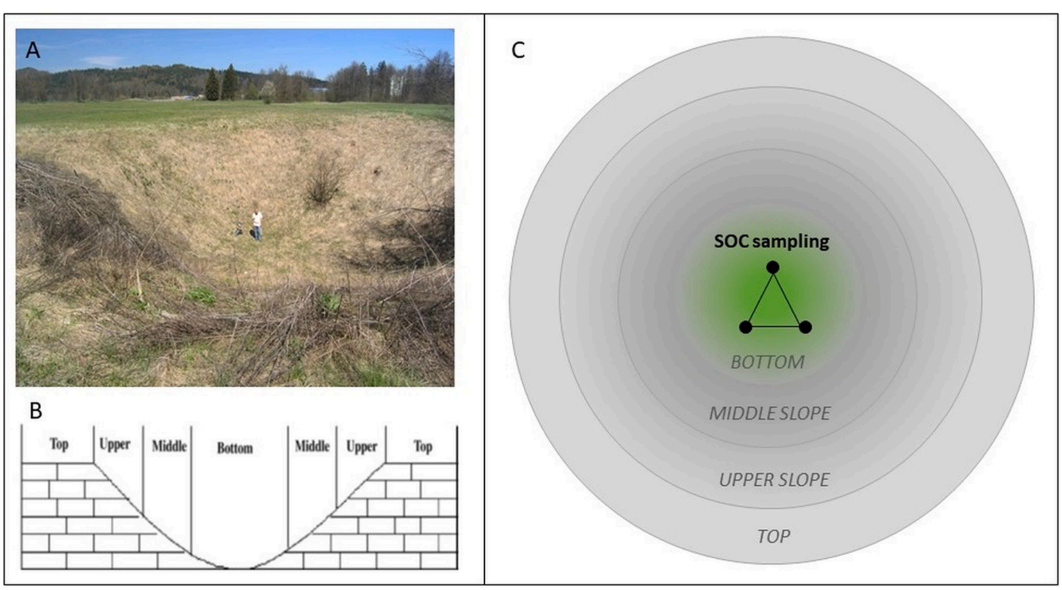

Fig. 2. A landscape photography of concave doline landform (A), simplified cross-section with different topographic units (B) of dolines (adapted from Li et al., 2008) and simplified SOC sampling scheme (C).

Samples were placed in plastic bags with an identification card and brought to the laboratory the same day (Laboratory for Forest Ecology, Slovenian Forestry Institute). Each soil sample was given a unique laboratory number and air dried. Samples were crushed and sieved at the same time (ISO 11464), using a de-agglomerator Fritsch Pulverisette 8 through a 2-mm sieve. Roots and stones thicker than 2 mm were removed. The roots were dried at 105 °C for 24 h and weighed. The volume of roots was calculated from their mass and average density (0.8 kg dm^3). The stones were weighed and their volume was measured.

The following analyses were performed on all samples: Moisture content (at 105 °C, ISO 11465) using Sartorius MA45 moisture metre (Sartorius, Goettingen, Germany), pH in 0.01 M $CaCl_2$ solution (ISO 10390) using Metrohm automatic pH metre (Titrator 702 SM Titrino with Autosampler 778 Sample Processor, Metrohm, Herisau, Switzerland), total carbon (ISO 10694) and total nitrogen (ISO 13878), both at a temperature of 1140 °C on the CNS Elementar vario MAX cube analyzer (Elementar, Langenselbold, Germany). When the pH of the sample was higher than 6.0, carbonate analysis (ISO 10693) was performed using a calcimeter (Scheibler instrument, Eijkelkamp Soil & Water, Giesbeek, The Netherlands). The determined mineral carbon content was subtracted from the total carbon value to obtain only the organic carbon. The results of total nitrogen and total, organic and mineral carbon were corrected to dry weight (105 °C). Texture analysis (ISO 11277) was performed on samples from the upper layer (0–10 cm), where we expected the greatest variability. The bulk density of the fine soil (BD in kg/dm^3) was calculated by dividing the dry mass of fine soil collected with the corer (kg) by the volume of undisturbed fine soil (dm^3). The volume of fine soil is the volume of the entire sample, minus the volume of rocks and roots. SOC stocks ($kg/m^2 \times 10 = t/ha$) were calculated as the product of the concentration SOC (g/kg), the BDfe (kg/dm^3) and the depth of the soil horizon (m), and the correction factor for stoniness (CFr = 100 - (% stones) / 100). The main statistical parameters were evaluated using the Statistica programme (TIBCO Software Inc., 2018). Statistical parameters such as mean and standard deviation (SD) of SOC, nitrogen (N), organic carbon/nitrogen ratio (C/N), texture, $CaCo_3$ (%) and pH at a depth of 0–40 cm were analysed using *t*-test. Multiple comparisons for all groups (ANOVA) were used to examine the effects of land use on SOC, N, texture (% sand, % clay) and pH. Mean comparisons were made using Kruskal-Wallis test at the p 0.05 level.

2.3. Land use analysis

Current land use and vegetation cover was assessed during the 2018 growing season during a field walk concurrent with soil sampling. A detailed historical land use change analysis was also performed for the last two centuries at the level of 22 studied dolines by comparing the 19th century Franciscan land cadastre (Franciscan Cadastre, 1823) and more recent aerial photographs (GURS, 2019). Additionally, to recognize the ongoing trend in land use changes throughout the Kras Plateau, the 2002 and 2020 Slovenian Land Use Maps (MKGP, 2020) were compared at the dolines landform level, by analysing over 14,000 dolines. For each year, total coverage of individual land use type was summed. Based on coverage (hectares) and average SOC storage, we made estimation of how much C was stored in dolines in Kras Plateau for each land use type in each year. Changes in SOC storage that are related to land use changes were determined.

3. Results

3.1. Calculated soil organic carbon in dolines under different land uses

This section presents general remarks on measured and calculated SOC stock capacity in dolines. The statistical analyses for SOC (stock in t/ha and concentration in g/kg), nitrogen (N), C/N ratio, reaction and the amount of fine-grained soil fractions is shown in Table 1. The SOC stock is summed for all four depths at the individual sampling site level, and the concentration of SOC is calculated as the mean value for the entire 40-cm layer. The mean SOC stock in the Luvisols in dolines is 116.4 t/ha. The interval between the minimum stock (85.8 t/ha) and the maximum (172.1 t/ha) exceeds 80 t/ha and shows big differences in storage capacity and influencing factors. SOM is represented also by nitrogen (N) and C/N ratio mean values, 0.27 % and 11.30, respectively. In 10 cm topsoil, fine silt is a prevailing fraction (ranged between 34.73 % and 50.60 %, with an average of 41.92 %), followed by clay (ranged between 20.78 % and 47.39 %, with an average of 34.44 %). In total, silt fraction amounts to over half of total soil fraction (56.2 %), while fine fractions bellow 0.02 mm account for over two-thirds (76.5 %) of soil.

The highest mean SOC stock is in succession plots (130 t/ha; 35 g/kg) (Table 1, Fig. 3A) and show tendency in decreasing SOC contents in order of young succession > forest > grassland. We detected measurable differences between the minimum and maximum SOC stock value in succession type, which indirectly shows the greatest dynamics within this type due to differences in age of succession. On contrary, the standard deviation of SOC values for grasslands and forests is lower, due to stabilized vegetation and ecosystem as well as SOC stock. Both, the mean concentration and the carbon stock are higher in the forest than in the meadow and are higher than 25 g/kg and 100 t/ha, respectively.

On average, SOC decreases with soil depth under different land use types (Fig. 4A, 4B). The highest mean N value was found in succession plots, while the lowest mean percentage was obtained in forest plots (Fig. 4C). The results for the same land use plots show significant differences in N content within forest and succession plots. The amount of N under different land uses decreased in the order of succession > grassland > forest. We also found significant differences in C/N ratio among land uses (Fig. 4D). It was lowest in grassland soils (C/N = 10.15). The mean C/N ratios among the different land uses were in the order forest > succession > grassland, but not significant.

Differences in soil reaction due to land use also appeared (4E). The highest mean values were measured in the soils of dolines with the land use of succession and grassland. On average, mean pH values decreased in the order of succession = grassland > forest, where also C/N is the highest (Fig. 4D). The highest mean share of clay content was measured in the soils of dolines covered with forests (41.06 %) (Fig. 4F, Table 1). Clay values decreased in the order of forest > succession > grassland.

Table 1
Basic statistics for soil organic carbon stock (t/ha/40 cm), SOC concentration (g/kg), total nitrogen (TN), C/N ratio, soil reaction (pH) and fine soil fractions in dolines bottom.

	Soil sampled in 22 dolines						
	All dolines				Dolines classified by land use		
	N	**Mean**	**Std. Dev.**	**Coef. Variance**	**Grassland (7)**	**Succession (6)**	**Forest (9)**
SOC (t/ha/40 cm) *per location*	22	116.42	22.21	19.08	106	130	116.64
SOC (g/kg) *per location*	22	30.25/*48.51* [10]	6.68	22.07	27.06/ *43.68* [10]	35.24/ *53.04* [10]	*29.41/ 48.82* [10]
TN (%)	88	0.27	0.12	43.87	0.26	0.27	*0.35* [10]
C/N	88	11.30	1.48	13.12	10.6	11.8	*13.6* [10]
pH (0.01 M $CaCl_2$)	88 *22*	6.17/ *5.77* [10]	0.77	12.60	6.63/ *6,22* [10]	6.4/ *5.92* [10]	*5.5/* *5.19* [10]
Clay < 0.002 mm (%)	22	*34.44* [10]	*8.56* [10]	*24.84* [10]	*26.45* [10]	*36.41* [10]	*39.32* [10]
Fine silt 0.02–0.002 mm (%)	22	*41.92* [10]	*4.25* [10]	*10.13* [10]	*39.81* [10]	*44.1* [10]	*42.1* [10]
Coarse silt 0,063–0,02 mm (%)	22	*14.28* [10]	*3.09* [10]	*21.62* [10]	*16.19* [10]	*12.88* [10]	*13.7* [10]

[10] Sample data for upper 10 cm soil layer.

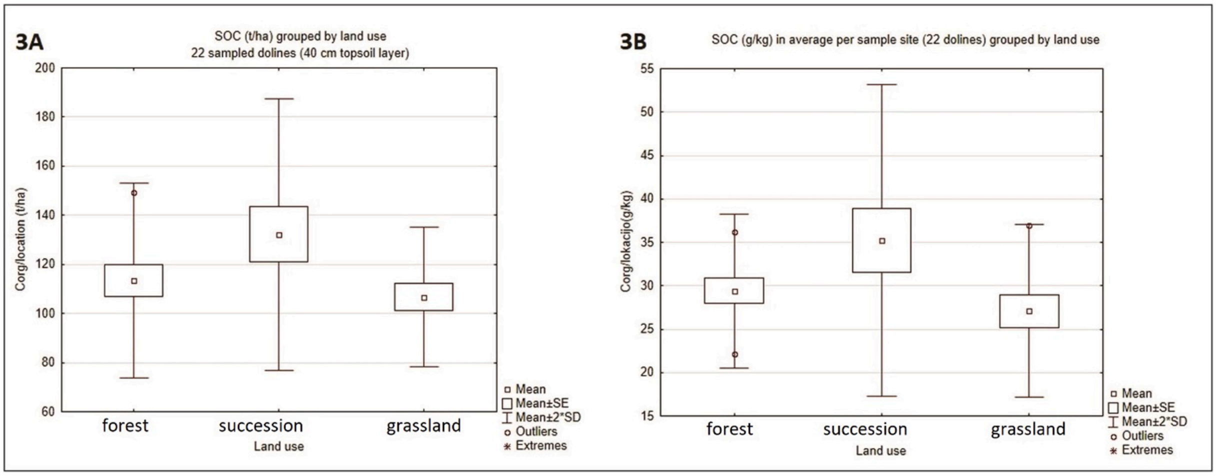

Fig. 3. SOC stock (t/ha/40 cm) (3A) and concentration (g/kg) (3B) in the upper 40 cm soil layer per location, and grouped by land use (N22: grassland 7, succession 6, forest 9). Graphs show no significant differences in SOC stock but a decreasing values in the order of land use: succession > forest > grassland.

3.2. Land use changes in dolines over the last 200 years

Based on historical land cadastre (Fransicesan Cadastre, 1823) and land use change analysis in 22 dolines it is evident that 85 % of today's permanent grassland plots were cultivated in the time of greatest agricultural expansion in Kras Plateau (19th century) (Table 2). They express the lowest SOC, and confirm that the most intensively cultivated plots are the most exhausted. In opposite, plots that were in the 19th century used as pastures or meadows have on average the greatest mean SOC stock in today's forest or succession plots.

By analysing land use in over 14,000 dolines (equal to 2,680.43 ha) of Kras Plateau (42,900 ha) between 2002 and 2020, the on-going trend of abandonment of cultivated dolines and natural afforestation was recognized (Table 3). SOC storage for year 2002 and 2020 were estimated for forest, grassland and succession dolines. The SOC storage was for 12,538 t/ha/40 cm higher in year 2002 in comparison to 2020. The most alarming is decrease of grasslands for 160 ha and increase of urban area for 110 ha. Although grasslands showed the lowest SOC storage, their contribution to total SOC storage in dolines is very important.

4. Discussion

4.1. Main characteristics of doline soils important for SOC storage

Through the study we examined three factors playing the main role in the SOC storage capacity in dolines: the amount of fine-grained soil fractions (clay, silt), soil thickness (leaching of clays and redistribution along soil profile), and the C/N ratio (related to intensity of land use).

Soils of dolines are fine-grained (76.5 % of fraction smaller than 0.02 mm) and accumulated in thick layers on the bottom of karst dolines. Most studies exposed mostly clay fraction in the soil (Yang et al. 2019), but we found, that one of the main factors for higher SOC storage in soils overlying carbonate bedrock (limestone) is the content of fine-grained fractions in total (clay, fine silt). As explained by previous studies (Eusterhues et al. 2005; Wang et al. 2013; Eze et al. 2018) clay minerals and iron oxides, among other oxides, play a key role in maintaining SOC in the stabilization and accumulation of SOC.

We found general differences in clay content (10 cm cover layer) between land use types (Fig. 4F). The highest average clay content was measured in the soils (10 cm layer) of dolines covered with forests (39.32 %), with a decreasing trend towards succession (36.41 %) and grasslands (26.45 %). Since clay formation is a long process, we assume that the clay particles were transported there also by wind and water from the neighbouring area and that the process of leaching was more disturbed by the dense vegetation than in soils covered with shrubs and grassland. Silt (fine and coarse) is also an important soil fraction in doline Luvisols (10 cm). They contain more than half (56.2 %) silt, regardless of land use (55.8 % in forest, 56.98 % in succession plots, 56 % in grassland plots).

Differences in soil reaction (pH) due to land use also occurred (Fig. 4E). The highest mean values were measured in the soils of dolines with the land use of succession and grassland. On average, mean pH values decreased in the order succession = grassland > forest, where C/N values were also highest (Fig. 4D). The highest mean clay content was measured in the soils of dolines covered with forest (41.06 %) (Fig. 4F, Table 1). The clay values decreased in the order of forest > succession > grassland. There may be some recent explanations for this observation. Changes in soil pH appear to be due to vegetation (Table 1, forest soils are most acidic, grassland least acidic). Acidic soil environment promotes the dissolution of limestone, which in turn leaves clay minerals as residue. On the other hand, certain vegetation types (e.g. grasslands, croplands) are better able to bind dust from the air (windblown dust), which could also contribute to the clay content of the soil.

When analyzing the storage of SOC in extremely thick doline soils (over 15 m) in relation to clay minerals, the distance from the parent material must be taken into account, as the parent material has a significant influence on SOC (e.g., Turrión et al., 2009; Fantappiè et al., 2010; Albaladejo et al., 2012). In our study, a higher SOC inventory in dolines may be influenced by a lack of calcium extracted from the parent material through deep soil horizons, which inhibits SOM decomposition (Čarni et al., 2007). In some dolines, sediments can be up to 15 m thick (Habič, 1978), so the upper layers of the Luvisols are not as affected by weathering processes of the parent material as the shallower Rendzic Leptosol (on slopes) and Chromic Cambisol outside dolines on apartment plateaus. Most studies report a decrease in SOC with soil depth, but the studies on SOC stock report total inventory increases when the thicker total soil layer is sampled for SOC storage (Patton et al., 2019). Estimated SOC stocks at 0.05 m, 0.1 m, 0.2 m, 0.3 m, 0.5 m, and 1 m depths cumulatively represent 11.2, 20.8, 37.0, 48.4, 66.0, and 90.8 % of the total SOC stock of the watershed, respectively, considering only a 1-m layer (Patton et al., 2019).

According to the results of the 22 doline plots studied, the top 40 cm thick soil layer in the dolines stores on average over 116,4 t/ha/40 cm (11.6 kg/m^2 in 0–0.4 m layer) SOC (Table 1). The formation of soils and soil processes at this location is greatly influenced by the "landscape topography" within the doline as well as the rest of the landscape surrounding the doline that drains into the doline. The surrounding landscape contributes to what is happening in the doline. Leached soils that form in dolines are due to the dolines being points in the karst landscape where surface runoff is concentrated, concentrating eroded soil

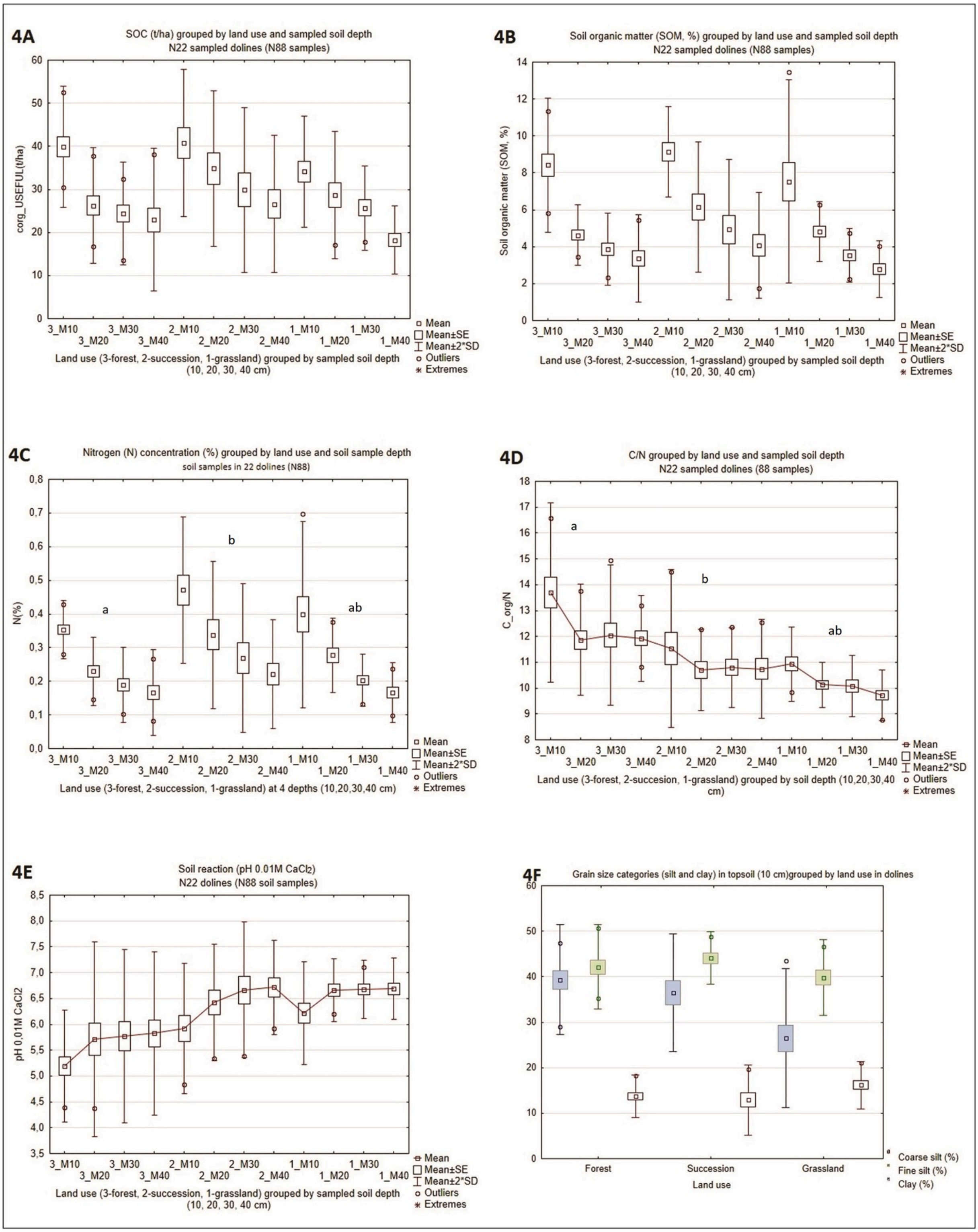

Fig. 4. Distribution of soil organic carbon (SOC) stock (t/ha) (4A), soil organic matter (SOM) (%) (4B), soil nitrogen (N)(4C), C/N ratio (4D), soil reaction (4E) and soil texture (% of soil particles) (4F) along with different land uses in observed dolines. The letters (a, b, ab) denote homogeneous groups of values at a 0.05 significance level. Groups with the different letters are significantly different from each other; ab – the group is neither different from a and b.

sediments and SOC on well-defined areas of the land surface. Reference studies on SOC stands in dolines are lacking. A discussion of the results can be made by comparing SOC stocks at the level of concave landforms in fluvial geomorphological systems. Patton et al. (2019) examined variations in measured soil thickness and organic carbon content with concave topography by analysing a 1 m thick topsoil layer. Concave topography comprised 61 % of the total catchment inventory SOC (22.5 kg/m^2 in the 0–1 m layer), and concave areas contained significantly greater total profile SOC stocks than divergent regions (24.9 compared to 3.89 kg/m^2 in the 0–1 m layer). In addition, many other studies (e.g., Sanderman et al., 2009; Kunkel et al., 2011; Lybrand and Rasmussen, 2015) have also shown greater total abundance of the SOC profile in

Table 2
Land use changes in 22 studied dolines examined for years: 1823 and 2018.

SITE CODE	SOC (t/ha/40 cm)	LAND USE 2018	LAND USE 19th century
LD04	111,67	grassland	cropland
VT03	125,21	grassland	cropland
MKD02	106,90	grassland	cropland
MKD03	119,70	grassland	cropland
VT04	85,81	grassland	cropland
LD03	106,59	grassland	cropland
LD01	91,24	grassland	grassland
KD03	132,92	succession	grassland
KD07	150,95	succession	cropland
ZV4	133,10	succession	grassland
ZV5	103,36	succession	grassland
KD06	100,25	succession	grassland
ZV14	172,14	succession	grassland
GV04	115,16	forest	cropland
KD12	127,19	forest	grassland
GO3	149,15	forest	grassland
GV02	103,34	forest	grassland
GV03	87,97	forest	grassland
KD08	122,07	forest	grassland
KD09	98,50	forest	grassland
KD10	126,04	forest	grassland
KD13	91,94	forest	grassland

Table 3
Trend in land use changes in over 14,000 dolines of Kras Plateau between 2002/2020.

LAND USE TYPE	Land use type coverage (ha) in years 2002 and 2020			SOC storage in 14,000 dolines t/ha/40 cm		
	2002	2020	Difference 2002/2020	Average value	SOC in 2002	SOC in 2020
Forest	1,645	1680	35	116	190,820	194,880
Grassland	719.1	558	−161	106	76,214	59,148
Succession	190.4	194	3.6	130	24,752	25,220
Cropland	86.2	76,5	−5.2	/	/	/
Urban-land	33.7	144	110.3	/	/	/
Abandoned	0.00	23	23	/	/	/
Other	5.9	4,5	−1.4	/	/	/
Total land use in dolines	**2,680**	**2,680**	/	/	**291,786**	**279,248**

concave compared to divergent regions. To date, there are no studies on SOC storage (t/ha) in soils of Dinaric karst plateaus. Comparison with available studies (e.g., Yang et al., 2019; Ogrinc et al., 2016) is possible only for SOC content (g/kg) in loose soil and at comparable soil thickness. The mean SOC -content (Fig. 1) in topsoil (0–40 cm of dolines) is 30.25 g/kg and the values ranged from 20.44 to 45.00 g/kg and 29.41 g/kg in forest soils, respectively, while the content in forest soils on the plateau was lower (21.2 g/kg). The average SOC content in the upper 10 cm of the soil layer in forest dolines (48.82 g/kg) was also higher than in plots outside the forest (44.95 g/kg).

The average SOC in dolines was compared with soil samples from other areas with carbonate bedrock in Slovenia (one sample Chromic Cambisol and one Rendzic Leptosol). The SOC content ranged from 12.1 to 45.5 g/kg, with an average value of 22.9 g/kg at four depths (0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm) (Yang et. al., 2019), and it is lower than in dolines. With regard to soils developed on carbonate rocks, Šamonil (2007) reported high SOC concentrations of 45.9 g/kg (average value for 0–50 cm depth) in Rendzic Leptosol and between 34.9 g/kg (0–9 cm depth) and 11.2 g/kg (9–52 cm depth) in Calcaric Cambisol under forest fragments, including sections of Bohemian Karst. However, this is not representative of carbon storage (t/ha).

4.2. Soil organic carbon in dolines under different land uses

4.2.1. Soil organic carbon storage in grassland-dolines

The traditional land use in the Kras Plateau was agriculture. Plots in dolines were used as croplands, meadows and pastures (Table 2). Traditionally cultivated meadows are elaborated. Grassland dolines are mostly typical semi-natural mesic meadows on deep fertile soils found on karst plateaus throughout Europe. These meadows are fertilized and mowed 2–3 times per year (Breg Valjavec et al., 2017; Škvorc et al., 2020). The predominant species are eutrophic grasses and herbs, such as *Arrhenatherum elatius, Poa pratensis, Taraxacum officinale*, etc. Farmers apply fertilizer (N) to the grasslands and harvest plant biomass OC as hay from these ecosystems. As a result, SOC is low in these grassland covered dolines. 85 % of the current permanent grassland was cultivated during the period of greatest agricultural expansion at Kras Plateau (19th century). The land was used to grow vegetables for the nearby port cities (e.g. Trieste). It is not surprising that the lowest population of SOC was measured in the former croplands, which are now a permanent meadow. This is generally consistent with other studies of SOC stock in abandoned agricultural lands (e.g. cultivated terraces) (Djuma et al., 2020). The most intensively cultivated plots are the most depleted. The impact of historical land use can have long-term impact on SOC stock and stabilization. According to previous studies (e.g. Kurganova and Lopes de Gerenyu, 2008) long-term changes in land use do affect the ratio between the labile and recalcitrant SOC fractions, with a marked increase in recalcitrant SOC in the 77 years following land use change from cropland to permanent grassland.

4.2.2. Soil organic carbon storage in succession-dolines

Succession dolines are the best SOC pools when succession follows cropland or intensively fertilised meadows, as in case of studied dolines (for location see Fig. 1). When traditional management is abandoned, succession towards shrub and forest communities begins (Breg Valjavec et al., 2018b). Today's succession plots in dolines are covered by shrub communities dominated by *Prunus spinosa*, forming dense communities. These communities are typical of abandoned meadows and croplands with high levels of remaining nutrients. This stage occurs rapidly after abandonment and lasts for many years until nutrient availability declines and more successional species appear (Čarni, 1998). In these stands, many nitrophilous and ruderal species occur, such as *Elymus repens*, *Galium aparine*, *Vicia hirsuta*, accompanied by species of the former land use (*Lathyrus pratensis, Trifolium pratense, Vicia sepium*) and representatives of the future successional stages (*Crataegus monogyna, Euonymus europea, Ligustum vulgare*). In this primary succession stage, there is no more additional nutrient input, which decreases the total nutrient content (N) and increases the C/N ratio. This process simultaneously increases organic matter in permanent grassland ecosystems and SOC. This phase may continue for some time until nutrient availability is high. When nutrient levels decrease, tree species may take over the dominant role (Čarni, 1998).

4.2.3. Soil organic carbon storage in forest-dolines

In sustainably managed forests, the SOC stock is usually higher than in croplands and meadows, but in the case of succession in dolines (especially young succession), these are better SOC pools. Similar to grassland dolines, today's forested dolines of Kras Plateau are still impacted by past agricultural land use. All studied dolines were in 19th century used as grasslands (Table 2), and were all agriculturally abandoned before year 2002. Our vegetation survey revealed these are at least 50 years old forests. They started to form after artificial and natural afforestation in the first half of 20th century in parallel with the abandonment of agricultural land (Kaligarič and Ivanjšič, 2014; Breg Valjavec et al. 2018b). Present-day forests in dolines are dominated by *Quercus petrea, Quecus cerris* and *Carpinus betulus*. Most of the communities in our case were also (co-)dominated by *Corylus avellana. Corylus avellana* forms (pre-)forest communities, often 5–10 m high, occurring between shrubs and established forests (Poldini, 1989; Čarni, 1997; Dakskobler 2006; Dakskobler et al., 2017). Typical forest floor species are already present, such as *Asarum europaeum, Cyclamen europaeum, Primula vulgaris, Salvia glutinosa, Viola reichenbachina* and others.

4.3. Effect of land use change on today's SOC storage in dolines

Considering preliminary prediction, based on literature, that the highest levels of SOC stock would be found in forests land use (due to high litterfall) proved to be incorrect. The explanation for this phenomenon is quite complex. We must start with the discussion thousands of years ago. Kras Plateau landscape identity was formed in the last two millennia and suffered from heavy deforestation, erosion and almost karst rocky desertification (Kaligarič and Ivanjšič, 2014). In the 20th century, artificial and natural afforestation began in parallel with the abandonment of agricultural land (Breg Valjavec et al. 2018b). Natural succession towards afforestation is still ongoing (Kaligarič and Ivanjšič, 2014). By analysing recent land use change trend in 14,000 dolines of Kras Plateau (429 km^2) we proved the on-going succession toward forest also in dolines. Considering the recent trend of agricultural land abandonment in the Kras Plateau generally even more afforestation of dolines, and consequently an increase of SOC stocks in their soils can be expected. Another threat to SOC doline pools is un-controlled and spreading urbanisation. Comparison of SOC storage for years 2002 and 2020 showed for 12,538 t/ha/40 cm higher SOC stock in dolines in 2002 in comparison to 2020. The most alarming is decrease of grasslands for 160 ha and increase of urban area for 110 ha. Although grasslands showed the lowest SOC storage, their contribution to total SOC storage in dolines is very important. In continuation, additional study needs to be conducted at the national level, since there are over 470,000 dolines mapped in Slovenia (Mihevc and Mihevc, 2021) and exposed to different land use practices.

Beside land use changes, also climate change poses a significant problem for the future of soil carbon stocks in dolines due to changing conditions for plant production and decomposition. Albaladejo et al. (2012) emphasize that climate change could lead to significant shifts in the nature and distribution of land use, affecting both carbon sequestration and the overall regional carbon budget. Ostle et al. (2009) refer to numerous studies from the UK (e.g. Freeman et al., 2001; Briones et al., 2007; Sowerby et al., 2008; Harrison et al. 2008) which indicate that organic or carbon-rich soils, including peatlands, rankers, peaty podsols, stagnogleys and brown soils, are most likely to be most sensitive to climate change. In this case, the microclimate in dolines will also change.

Increasing the stable SOC is one of the milestones for a resource-efficient Europe (Panagos et al., 2013). Estimating the SOC stock is important from the perspective of Kyoto Protocol (2005) and currently Paris Convention to achieve a balance between anthropogenic emissions by sources and removals by sinks of greenhouse gasses as well as organic carbon sinks. The patterns and dynamics of carbon storage in ecosystems following land use change are therefore of particular interest for estimating soil carbon gains and losses at regional scales (Wilson and Puri 2001; Zhang et al. 2007). The SOC measurements in dolines shows big differences in storage capacity and different influencing factors, mainly land use type. The interval between the minimum stock (85.8 t/ha) and the maximum (172.1 t/ha) exceeds 80 t/ha. We found measurable differences between the minimum and maximum SOC stock values in the succession type, indirectly showing the greatest dynamics within this type due to differences in succession age. In contrast, the standard deviation of the SOC values for grassland and forest is lower, which can be attributed to the stabilized vegetation and ecosystem as well as the SOC stock (Buringh, 1984). Both mean concentration and carbon stock are higher in forest than in grassland, exceeding 25 g/kg and 100 t/ha, respectively.

4.4. Future reserach

Karst landscapes cover about 8,800 km^2 or 44 % of the Slovenian territory. There are over 14,000 dolines (about 40/km^2) on Kras Plateau alone and over 470,000 solution dolines (>20/km^2) in Slovenia as a whole (Mihevc and Mihevc, 2021). Their potential for CO_2 sequestration needs further investigation by comparing different plateaus, for example with different climatic regions in the Dinaric Karst. Further in-depth sediment studies are needed for all doline topographic units (see Fig. 2C) to compare the differences inside of doline and to define the ongoing soil processes considering the transport and storage of soil organic matter with respect to soil inorganic matter. Dolines are effective soil traps. These soils are not only the result of karst dissolution, but they may originate from other processes: pedogenetic processes, weathering degradation, collapse, alluvial and eolian processes, and finally periglacial environmental processes (Sauro, 2009). In addition, the surrounding landscapes concentrate surface water runoff to the center of the dolines. The role of the surrounding slopes within the dolines needs to be considered in the future, as the concentrated runoff will carry erosional sediments, organic material, and particulate organic matter that will be deposited in the center of the dolines. This will affect the SOC of the dolines and create SOC gradients within the doline.

5. Conclusions

Studied karst dolines are representative landform of karst plateaus worldwide and they were recognized as potential SOC pools, based on case study from Kras Plateau of NW Dinaric Mountains in SW Slovenia. Due to specific soil characteristics (fine-grain fractions, deep soil layers, C/N ratio) dolines are hot-spots of Corg sink and therefore important in the context of land use changes as potential organic carbon pools. In Kras Plateau, the (agricultural) cultivated landscape has almost disappeared and natural process of afforestation is in progress. The semi-natural mesic grasslands inside of dolines are disappearing and this affects the potential for total storage capacity of dolines. The SOC storage was for 12,538 t/ha/40 cm higher in year 2002 in comparison to 2020 due to the alarming decrease of grassland-dolines for 160 ha and consequential increase of urban area spreading over additional 110 ha of doline landscape. Although grasslands showed the lowest SOC storage, their contribution to total SOC storage in dolines is very important. Further land use policies and conservation management should be done wisely to balance the abandonment of agricultural land with urbanization and natural afforestation while preserving and protecting the SOC pools in the dolines. It is also necessary to improve the understanding of dolines as possible SOC pools in many other karst plateaus in Europe (Mecsek/Hungary, Gargano Plateau/Italy, Gorski Kotar/Croatia, Causses/France, Crete/Greece, etc.) as well as in other continents (e.g. Japan/Akiyoshidai Plateau, North Ameica/Appalachians and South America/Brazil), where dolines present spatially widespread karst landforms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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