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Stable isotope composition in tree rings of *Fagus sylvatica* L. saplings reflects environmental variation induced by silviculture and microsite factors

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ABSTRACT

Natural regeneration of tree species is sensitive to silvicultural interventions. This study aimed to investigate the effects of different cutting intensities and local topographic and soil conditions on the composition of stable carbon (δ^{13}C) and oxygen (δ^{18}O) isotopes in wood of young beech (*Fagus sylvatica* L.) trees. Beech saplings in the regeneration layer were sampled in summer 2018 at three study sites in Dinaric fir-beech forests in the karst area of Slovenia. Three different cutting intensities were performed in 2012: i) no cutting (control), ii) 50% cutting of the stand's growing stock creating thinned stands, and iii) 100% cutting of the stand's growing stock creating 0.4 ha canopy gaps. We show that δ^{13}C increased along the gradient of cutting intensity. On average, δ^{13}C values in the tree rings were ~ 2‰ increased in trees from canopy gaps than from closed control stands. Furthermore, δ^{13}C was higher on south-facing slopes characterized by higher air temperatures and lower relative humidity compared to north-facing slopes of karst sinkholes. Additionally, the results suggest a dependence of δ^{18}O on interannual and cross-site climatic variations, particularly in the case of summer precipitation amount. δ^{18}O also responded to soil depth, with beech individuals exhibiting lower values on deeper soils, presumably characterized by higher soil water availability compared to shallow soils. The results are discussed in the context of future climate change, as many beech-dominated forests on karst terrain in the Dinaric Mountains are particularly affected by climate warming and drying due to prolonged and reoccurring summer droughts, intensified large-scale disturbances, and often shallow soils with low water storage capacity.

1. Introduction

The tree regeneration layer is an important component of the forest vegetation (Thom et al., 2022). Knowledge on eco-physiological responses of tree regeneration to management disturbances provides valuable insights for improved silvicultural methods to mitigate the effects of climate change (Di Matteo et al., 2017). Severe drought events and increasing frequency of large-scale forest disturbances have the potential to cause serious declines in the natural regeneration of key tree species. European beech (*Fagus sylvatica* L.) is known to be very sensitive to drought (Fotelli et al., 2009) and its natural regeneration is threatened under expected climate change scenarios (Fruleux et al., 2016). At the same time, beech exhibit considerable plasticity of leaf morphological and physiological traits in response to climatic and edaphic conditions (Weithmann et al., 2022), especially at younger phases as it can tolerate a broad range of understory environments and benefit from increased light availability following management activities or natural disturbances opening the tree layer canopy (Čater and Levanič, 2019; Kermavnar et al., 2021). Therefore, its sensitive response to environmental factors makes it suitable species for studying the eco-physiological effects of silvicultural treatments on the development of regeneration layer and post-disturbance succession, particularly in case of management systems where forest restoration is based on natural regeneration and release of advance regeneration (Čater and Diaci, 2017). In contrast to Central European beech ecosystems, little is known about the ecophysiology of beech in its transitional area between Central and South-eastern Europe (Fotelli et al., 2009).

Forest management practices, depending on intensity and spatial extent, cause significant alterations in the physiological and growth processes of tree regeneration layer in forest ecosystems by directly or indirectly modifying the availability of above- and below-ground resources. The most obvious change following tree cutting is in light

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availability due to increased radiation in canopy gaps compared to closed stands (Aussenac, 2000). Higher radiation results in pronounced alteration in microclimatic parameters, namely air temperature and relative humidity (Kermavnar et al., 2020). In forests with rugged terrain, spatial patterns in microclimate can be modulated by local topographic factors such as slope aspect and slope steepness. Higher mean and maximum air temperatures and lower relative humidity in canopy gaps lead to higher atmospheric vapor pressure deficit (VPD) compared to undisturbed stands (Kermavnar et al., 2020). This indicator of atmospheric aridity has been established as a major contributor to recent drought-induced plant mortality (Grossiord et al., 2020; Feng et al., 2022). Another key controlling factoring is soil water status. Similarly to high VPD, low levels of soil moisture availability force plants to decrease the degree of stomatal opening. Soil water availability may increase after stand thinning or canopy gap creation as the canopy rainfall interception and water uptake by trees are reduced (Di Matteo et al., 2010). However, these effects largely depend on site characteristics, e.g., soil depth and texture (Saurer et al., 1997). Trees respond physiologically to modifications in each of these resources with changes in the isotopic signatures recorded in tree rings (Marshall et al., 2022).

The stable isotopes of plant tissues can serve as an ecologically meaningful tracer of changes in the abiotic and biotic environment after disturbances (Dawson et al., 2002; Levanič et al., 2011; Cernusak et al., 2013). The composition of carbon isotopes ($\delta^{13}C$) and oxygen isotopes ($\delta^{18}O$) in the cellulose of tree-rings have been widely used to determine the physiological effect of different silvicultural practices (Di Matteo et al., 2017) and mechanisms responsible for responses of trees to extreme climatic events such as drought (Hartl-Meier et al., 2015). For example, Fotelli et al. (2003) demonstrated that the $\delta^{13}C$ of beech regeneration is indicative of recent environmental conditions in the forest understory. Similarly, Geßler et al. (2001) reported that the $\delta^{13}C$ signatures of beech trees reflect short-term fluctuations in water availability and intercepted radiation. Owing to its sensitivity towards variations in current environmental conditions, $\delta^{13}C$ of beech is a promising tool for evaluating the responses of beech regeneration to silvicultural interventions under conditions of global climate change (Fotelli et al., 2003). The eco-physiological mechanisms influencing tree-ring isotope composition are relatively well understood. However, only a few studies have simultaneously combined $\delta^{13}C$ and $\delta^{18}O$ analyses in silvicultural experiments (Di Matteo et al., 2017) although the dual isotope approach (Kruse et al., 2012; Siegwolf et al., 2022) can be beneficial for more detailed understanding of eco-physiological responses (Keitel et al., 2003; Roden and Farquhar, 2012; Lin et al., 2022).

According to carbon isotope theory, $\delta^{13}C$ in tree rings is controlled predominantly by two physiological components: i) stomatal conductance (g_s, a measure of the degree of stomatal pore opening, i.e., rates of CO_2 entering or water vapor exiting through the leaf stomata) during carbon fixation and ii) photosynthetic assimilation rate (A), both of which are influenced by a number of environmental factors, e.g., soil moisture, temperature, solar radiation and nutrient availability (Farquhar et al., 1989; Saurer et al., 1995; Warren et al., 2001; McCarroll and Loader, 2004; Vitali et al., 2021). Relative humidity and soil water content have a relatively direct effect on stomatal opening of plant leaves and consequently on $\delta^{13}C$ levels. Stomata are more closed under low precipitation and low humidity than under more humid conditions. Closure of the stomata prevents water loss, reduces g_s, and limits photosynthesis, resulting in increased (less negative) $\delta^{13}C$ values (Saurer et al., 1995; Saurer et al., 1997; Lauteri et al., 1997; Keitel et al., 2003). An increase in $\delta^{13}C$ may be the result of either reduced stomatal conductance (at constant A_{max}) or increased photosynthetic capacity (at constant g_s) (Scheidegger et al., 2000). Forest stand thinning and gap creation may improve soil water availability and remaining trees may respond by increasing stomatal conductance (Di Matteo et al., 2010; Powers et al., 2011). For this reason, some authors hypothesized and confirmed a decrease in $\delta^{13}C$ following thinning of mature stands (McDowell et al., 2003; Giuggiola et al., 2016; Di Matteo et al., 2017). Such effects, however, may not come to realisation in the response of tree juveniles in the shrub layer as competition for resources intensifies if canopy gaps are colonized by dense understory vegetation (Powers et al., 2011). At sites where water rather than light is limited, thinning also enhances soil evaporation and may not be beneficial (Giuggiola et al. 2016). Natural regeneration of beech may be inhibited by interference from competitors in the early-successional stage, especially during summer drought (Fotelli et al., 2001). Light and temperature are core determinants in plant photosynthetic rate and efficiency (Warren et al., 2001). Tree cutting leads to increase in light availability and air temperature (Aussenac, 2000; Fotelli et al., 2003). Leaves in light-rich environments tend to have higher light-saturated photosynthetic rates than those in shaded environments (Powers et al., 2011). The A/g_s ratio is positively correlated with $\delta^{13}C$ and represents intrinsic water-use efficiency (assimilated carbon vs. transpired water; Kruse et al., 2012; Di Matteo et al., 2017).

The oxygen isotope ratio ($\delta^{18}O$) is mainly determined by the composition of soil water (Lin et al., 2022). The $\delta^{18}O$ signature of tree-ring shares dependence on stomatal conductance with the $\delta^{13}C$ signature, while it is not directly related to CO_2 assimilation. The abiotic factors affecting $\delta^{18}O$ are expected to be different from those determining $\delta^{13}C$. In terms of climate signal, $\delta^{13}C$ has been shown to be strongly correlated with solar radiation and summer temperatures (Hilasvuori et al., 2009; Young et al., 2012) whereas the $\delta^{18}O$ signature in tree rings might depend more on interannual variability in precipitation across larger spatial scales (Marshall et al., 2007). For example, it has been shown to correlate with annual precipitation and summer rainfall amount (Young et al., 2015). Low precipitation totals generally result in enriched oxygen isotope ratios. Variations in $\delta^{18}O$ values should at least partly reflect evaporative demand of a plant (Keitel et al., 2006; Powers et al., 2011; Feng et al., 2022). Dry air and reduced soil moisture availability causes a decrease in g_s and an increase of $\delta^{18}O$ (Saurer et al., 1997; Scheidegger et al., 2000, Brooks and Mitchell, 2011; Lin et al., 2022).

Here, we assessed the $\delta^{13}C$ and $\delta^{18}O$ response of naturally regenerated beech saplings to various environmental parameters in the Dinaric beech-fir forests. The main ecological gradients were created by different cutting intensities. In addition, microsite factors not necessarily associated with silvicultural treatments were considered as well. We expected that the stable isotope composition of studied beech wood samples would be related to environmental differences caused by management disturbance. Along a gradient from closed forest stands to canopy gaps, we hypothesized that despite higher light availability, beech seedlings would be exposed to higher evaporative demand (VPD) in gaps. A prevailing prediction was that under hot and dry microclimatic conditions, when leaf stomata are often closed, $\delta^{13}C$ in wood cellulose would be increased (Kostić et al., 2022). Isotope composition of both elements was expected to be modulated by soil properties, a proxy for soil water status. It was also hypothesized that the predictors between $\delta^{13}C$ and $\delta^{18}O$ would be different and that $\delta^{18}O$ values would be less environmentally sensitive compared to $\delta^{13}C$. While much attention has been given to how persistent water scarcity alters forest composition and demography through increasing mortality rates (Gazol and Camarero, 2022), it is also crucial to quantify how these changes may impair tree regeneration. Such research questions are of high relevance to forestry, especially in the Dinaric region where disturbance regimes are shifting due to climate change (Nagel et al., 2017; Kutnar et al., 2021).

2. Materials and methods

2.1. Study area

The forests in the Dinaric Mountains are part of the largest forested complex in the Balkan Peninsula. The Dinaric fir-beech forests are one of the most widespread forest types in Slovenia, covering nearly 10% of the total forest area (Bončina et al., 2021). These uneven-aged forest stands in the montane vegetation belt are dominated by European beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.), often with an artificially increased proportion of Norway spruce (*Picea abies* Karst.). They occur on carbonate bedrock (limestone and dolomite), with numerous karst phenomena. Karst topography is diverse, shifting from limestone outcrops to numerous bowl-shaped terrain depressions (sinkholes), representing one of the dominant landforms in the Dinaric Mountains. Karst sinkholes vary greatly in dimensions and have important effect on tree growth (Kobal et al., 2015a), the diversity and composition of ground vegetation (Kobal et al., 2015b) and vegetation response to forest management (Kermavnar et al., 2021). Diverse relief is reflected in heterogeneous soil conditions, with development and physio-chemical properties of forest soils varying at small spatial scales (Kobal et al., 2015a; Kobal et al., 2015b). Soil has variable depth and is generally correlated with surface rockiness. The most common soil types are deep brown calcareous soils and shallow rendzinas. Due to the high percentage of skeletal material in the soil, soil texture is often not favourable for moisture retention, resulting in soils with low water-holding capacity.

This study was conducted at three sites in different parts of the distributional range of Dinaric fir-beech forests in Slovenia. The site “Rog” is located in the south-eastern part, the site “Snežnik” in the southern and the site “Trnovo” in the north-western part of the distributional range (Fig. 1). Altitude ranges from 750 m to 900 m a.s.l. and the study sites exhibit considerable differences in climatic conditions. Generally, the amount of annual precipitation in Slovenia decreases from west to east (Fig. 1c). Thus, the Rog site located in the south-eastern part receives less precipitation compared to the other sites, while the Trnovo site is under marginal influence of the Sub-Mediterranean climate (Table 1; Kutnar et al., 2015). The study period 2012–2018 was characterized by warmer and wetter annual averages but also hotter and drier summers compared to the 1989–2018 modern climate normal (Table 1).

Table 1
Characteristics of the three study sites in the Dinaric fir-beech forests, Slovenia. MAT – mean annual temperature, MST – mean summer temperature, MAP – mean total annual precipitation, MSP – mean total summer precipitation. Values are mean ± stand. deviation for two periods: modern climate normal expressing 30-year (1989–2018) average and our study period from 2012 to 2018. Climate data were compiled from the SloClim database (Škrk et al., 2021).

Site	Coordinates	Elevation [m]	Modern climate normal 1989 – 2018		Study period 2012 – 2018	
			MAT [°C] MST [°C]	MAP [mm] MSP [mm]	MAT [°C] MST [°C]	MAP [mm] MSP [mm
Rog	45.668° N, 15.033° E	831–902	8.3 ± 0.7	1384.3 ± 244.1	8.7 ± 0.4	1462.0 ± 225.7
			17.0 ± 1.0	376.4 ± 119.9	17.7 ± 0.8	314.3 ± 89.2
Snežnik	45.672° N, 14.460° E	753–815	9.0 ± 0.6	1469.3 ± 229.7	9.6 ± 0.4	1574.1 ± 332.2
			17.7 ± 1.0	333.7 ± 86.5	18.4 ± 0.4	288.5 ± 109.5
Trnovo	45.989° N, 13.759° E	801–869	8.8 ± 0.6	1870.5 ± 370.8	9.2 ± 0.4	2074.8 ± 394.4
			17.3 ± 0.9	387.6 ± 105.1	18.1 ± 0.8	356.2 ± 112.2

All three study areas are experiencing significant climate warming in last decades (Table 2). The highest change in mean annual temperature (MAT - daily mean) was calculated for the Snežnik site. The warming rate is even more extreme in summer months (MST - daily mean) and for maximum daily temperatures (MST - daily max). In contrast, MAP is declining. The decrease in summer precipitation is particularly evident at the Trnovo site (3.4% per decade). These trends indicate that the climate in the Dinaric forests in Slovenia is becoming warmer and drier. The presented data analysis is in line with meteorological observations

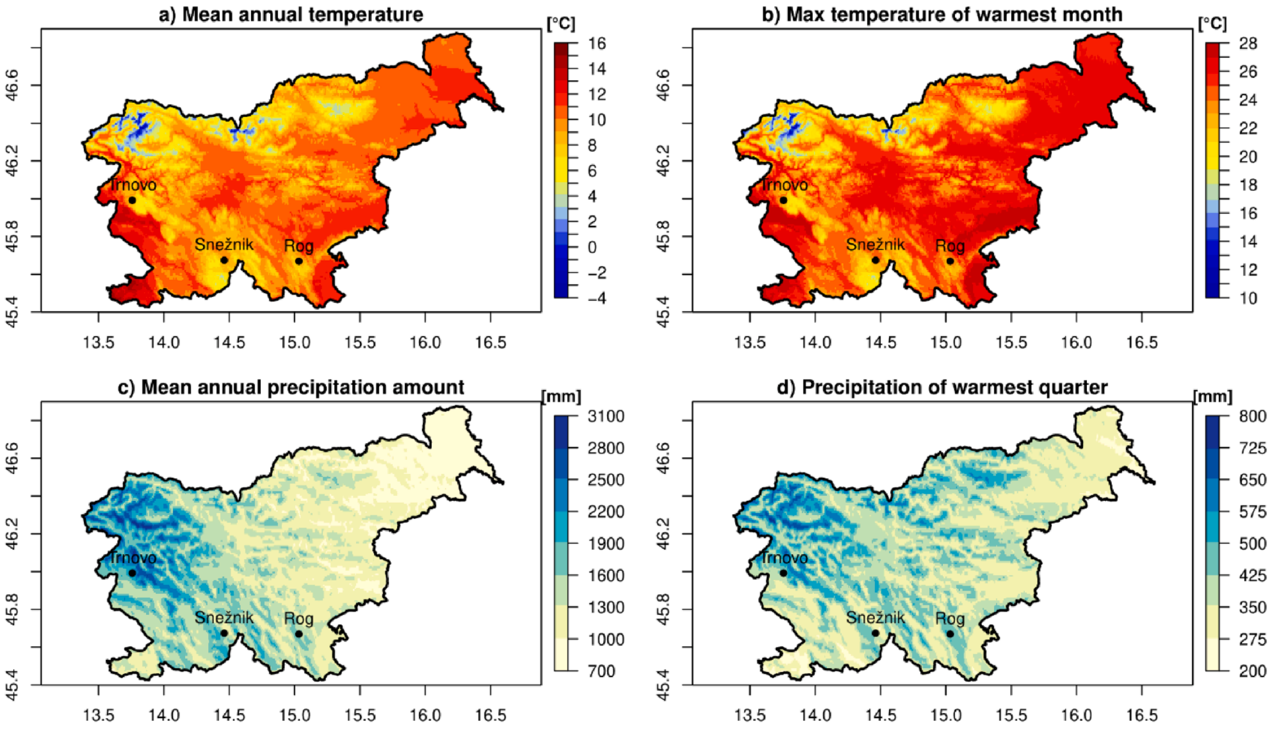

Fig. 1. Spatial maps with locations of the three study sites and four climatic parameters: a) mean annual air temperature [°C]; b) mean daily maximum air temperature [°C] of the warmest month (i.e., July); c) sum of the mean annual precipitation amount [mm]; d) sum of the mean precipitation amount [mm] of the warmest quarter (i.e., summer = June - August). Climatology for the period 1979–2013 was downloaded from CHELSA database version 1.2 (chelsa-climate.org; Karger et al., 2017).

Table 2

Long-term trends (1950–2018) in climatic parameters for each study site. Values are expressed as change per decade. Data were compiled from the SloClim database (Škrk et al., 2021). MAT – mean annual temperature, MST – mean summer temperature, MAP – mean total annual precipitation, MSP – mean total summer precipitation.

	MAT - daily mean (°C)	MAT - daily max (°C)	MST - daily mean (°C)	MST - daily max (°C)	MAP (%)	MSP (%)
Rog	0.23	0.25	0.34	0.39	−1.9	−0.8
Snežnik	0.29	0.28	0.39	0.40	−1.8	−1.7
Trnovo	0.19	0.28	0.29	0.42	−1.7	−3.4

of Slovenian Environment Agency (ARSO, 2022), reporting an increasing frequency of summer heat waves and prolonged droughts in the last decade.

2.2. Silvicultural experiment

At each study site, nine karst sinkholes of comparable dimensions were selected. In each sinkhole, a circular 0.4 ha plot was established with the centre at the bottom of the sinkhole. One third of the sinkholes was preserved as control stands in which no trees were cut. In one third of the sinkholes, 50% of the stand's growing stock was removed with the remaining trees being evenly distributed over the 0.4 ha experimental plot. The most intensive silvicultural measure was conducted in the last third of the sinkholes, where all trees were cut down, creating circular canopy gaps of 0.4 ha in size. Detailed information on our silvicultural experiment can be found in Kutnar et al. (2015), Eler et al. (2018) and Kermavnar et al. (2019a, 2019b, 2020).

2.3. Field sampling and samples processing

European beech (*Fagus sylvatica L.*) is the most abundant tree species in the natural regeneration layer of the Dinaric fir-beech forests (Bončina et al., 2021). Owing to its shade-tolerance, it can regenerate in closed stands with low light availability. However, after tree layer opening, this species shows high plasticity to changing environmental conditions and usually responds quickly with increased growth, sometimes even forming dense thickets. Sampling of young beech trees in the study plots was conducted in June and July 2018. Within each karst sinkhole, five young beech trees of similar height and age were systematically selected: one in the central part (bottom of the sinkhole), one in the northern part with south-facing aspect, one in the eastern part with west-facing aspect, one in the southern part with north-facing aspect and one in the western part with east-facing aspect. Sampled trees were more or less evenly distributed across the sampling area on all sides of the sinkhole. The total number of field sampled beech juveniles was 135 (27 × 5). Selected specimens of young beech trees with normal growth and vitality were cut with scissors at the base of the stem. The woody samples were put into cardboard boxes and then prepared for further processing in the laboratory.

Sampled stems of young beech trees were dried and sanded with progressively finer grade abrasive paper until the optimal surface resolution allowed the annual rings to be recognized and detected under magnification. Achieving a highly polished surface is particularly important in our case as beech is a diffuse-porous broadleaf species and we used young trees with small dimensions of woody stems. These were then scanned using a high-resolution ATRICS image capturing system (Levanič, 2007). The tree rings of each sample were dated, and each tree ring assigned to its exact year of formation. An overview of all sampled beech trees showed that the samples were a mixture of surviving advance regeneration (saplings older than 6 years) and newly established individuals (younger than 6 years).

2.4. Environmental factors

We collected data on estimated or measured variables that later served as explanatory predictors for the variation in stable isotope composition. During the field sampling of young beech trees in 2018, microsite slope aspect and local soil conditions were evaluated. Slope aspect was defined based on the position within the sinkhole, distinguishing between five categories (centre, north, east, south, west). Soil depth of the microsite was estimated using three categories: deep, intermediate and shallow. We estimated soil depth based mainly on surface rockiness, as these two are usually related (higher rockiness indicates shallower soil and vice-versa). Soil depth was used as a surrogate for soil moisture status, expecting deeper loamy soils would be moister whereas beech trees would experience greater drought stress on shallow skeletal soils.

Air temperature (T) and relative humidity (RH) were measured at three different within-sinkhole positions, i.e., in the centre, in the northern part (south-facing plots) and in the southern part of the sinkhole (north-facing plots). Continuous measurements over the growing season from May to October (a period with the strongest effect on isotopic variation; Saurer et al., 1997) with resolution of 30 min were available for 2012, 2013, and 2014. Based on T and RH, the vapor pressure deficit (VPD) was calculated using the following equation: $VPD = e_{sat} - e_{air}$,

where e_{sat} is the saturation vapor pressure and e_{air} is the actual vapor pressure (Murray, 1967; Thom et al., 2022). VPD is an ecophysiologically meaningful parameter. The fine resolution microclimate data were then aggregated to daily values. Details of the microclimatic measurements and derived parameters in our plots can be found in Kermavnar et al. (2020). Partial cutting, which retained 50% of the stand's growing stock, created a spatially heterogeneous mosaic of more open microsites with almost full light and more shaded microsites. Therefore, in the plots with 50% cutting, we also inspected the light conditions under which the young trees grew. Three categories were defined based on visual estimation of vertically projected tree layer (height above 5 m) canopy closure within the area with the radius of 1.5 m around sampled beech juvenile, using predefined scale: open (cover $< 33\%$), semi-shaded (cover between 33% and 67%) and shaded (cover $> 67\%$).

Long-term daily meteorological data (daily temperature and precipitation) were taken from the SloClim database (Škrk et al., 2021) for the period 1950–2018. These variables served as macroclimatic parameters varying interannually and between study sites.

2.5. Sample preparation and determination of the stable isotope composition

The tree-ring slicing was done by hand using a scalpel and stereomicroscope. A total of 512 tree-rings were extracted. Tree-rings between 2012 and 2018 were extracted as single ring, while tree-rings before 2012 were extracted as a single block. This decision was made because tree-rings in the vicinity of the pith were very narrow (<0.1 mm), that it was impossible to extract single tree-ring. Each sample tree-ring was put into a marked vial and transferred to the laboratory for α-cellulose extraction using method described by Loader et al. (1997). After cellulose extraction, all samples were homogenised using ultrasonic probe (Hielscher, Germany), then put overnight into a freezer at −18 °C and finally dried using freeze dryer at −90 °C under very low pressure (<1 mbar). Samples (0.175 ± 0.020 mg) for simultaneous determination of carbon and oxygen isotope were then weighted into silver capsules using a micro-analytical balance Sartorius CPA2P and analysed with Vario Pyro Cube Elemental Analyser (Elementar, Germany) running in a pyrolysis mode (1450 °C) and coupled with IsoPrime100 Isotope Ratio Mass Spectrometer (IsoPrime, UK).

Both certified reference materials (IAEA-601 and IAEA-602 for oxygen, and IAEA-CH3 for carbon) and in-house working standards (spruce cellulose, benzoic acid and corn flour) were used to control the accuracy and precision of the measurements. The analytical precision was < 0.1 ‰ for carbon and nitrogen, and < 0.3 ‰ for oxygen (expressed as a standard deviation of repeated measurements of in-house working standards (n = 30)).

Stable isotope results ($\delta^{13}C$ and $\delta^{18}O$) are reported in terms of relative delta (δ) value as a difference between $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratio of the sample and international reference material (Vienna PeeDee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW)) and expressed in per mil (‰) notation. All $\delta^{13}C$ and $\delta^{18}O$ analyses were carried out at the Stable Isotope Laboratory of the Slovenian Forestry Institute (Ljubljana, Slovenia).

2.6. Data analysis

All statistical analyses were performed in R software version 4.1.1 (R Core Team, 2021). Using a total of 512 tree-ring samples, the dependence of response variables ($\delta^{13}C$ and $\delta^{18}O$) was explored using a general linear mixed-effect modelling procedure incorporated in the *nlme* package (Pinheiro et al., 2022). Five categorical predictors were included as fixed effects: silvicultural treatment, study site, tree ring, aspect, and soil depth. Due to the hierarchical structure of our dataset, the random effects were plot (sinkhole) and ID of beech sapling (trees nested within sinkholes). The initial global model with all five predictors was built using the "dredge" function in the *MuMIn* package (Bartoń, 2022). This procedure results in automated model selection based on the corrected Akaike Information Criteria (AIC_c) for small sample sizes. Model diagnostics were checked graphically by looking at the distribution of fitted values vs. standardized residuals and the distribution of intercepts for random effects. In both cases, no evident trends were detected, indicating no significant assumption violation and sufficient model performance. The distribution of $\delta^{13}C$ and $\delta^{18}O$ values across the significant predictors was visualized by density plots (ridgeline chart) in the *ggplot2* package (Wickham, 2016).

Furthermore, we performed additional analyses for the relationship between $\delta^{13}C$ values and potential environmental predictors. Differences between categories of light conditions in the 50% cutting intensity in 2018 (n = 38) were tested with the non-parametric Kruskal-Wallis rank sum test using the *agricolae* package (de Mandiburu, 2020) and visualized with boxplots. We performed simple linear regression calculations correlating the $\delta^{13}C$ and the $\delta^{18}O$ data with microclimatic parameters (VPD_{max}) and several macroclimatic parameters: mean temperatures for individual month (April-October), mean temperature and precipitation amount in summer (June-August), mean annual temperature and precipitation. These relationships were plotted on a scatter diagram with linear trend line. The analysis for VPD_{max} included data for 2012, 2013, and 2014 (n = 39). In the case of macroclimate, the averaged data over three study sites and seven years (2012–2018) were used, resulting in 21 datapoints.

3. Results

3.1. Environmental factors explaining carbon isotope composition

The $\delta^{13}C$ data ranged from –32.6‰ to −24.6‰. The final linear mixed-effect model for $\delta^{13}C$ (AIC_c = 1413.5) included four significant predictors: silvicultural treatment, study site, tree ring, and aspect (Table 3), while soil depth was not found to be significant. Based on the model estimates, it was evident that treatment intensity had the largest impact on $\delta^{13}C$ values. On average across all years, the largest difference (almost 2‰) was between the control and the 100% cutting intensity (Fig. 2). The $\delta^{13}C$ values from the 50% cutting intensity were on average closer to the 100% cutting (difference in overall mean: 0.77‰) than to the control treatment (mean difference: 1.18‰). Both treatments were significantly different from the control (Table 3, Fig. 2).

Table 3
Fixed effects in the final model for $\delta^{13}C$ with four significant categorical predictors (treatment, study site, tree-ring and aspect). The following categories were used as references for coefficient estimates: control for treatment, Rog for study site, pre-treatment ring (tree-rings before 2012 extracted as a single block) for tree ring and central position for aspect. Data analysis was based on 512 samples (tree rings) extracted from 135 sampled beech juveniles.

	Estimate	Std. error	DF	t-value	p-value
Intercept	−30.357	0.29	371	−103.4	<0.001
Treatment 50% cut	1.184	0.28	22	4.3	<0.001
Treatment 100% cut	1.955	0.27	22	7.1	<0.001
Site Snežnik	1.217	0.27	22	4.5	<0.001
Site Trnovo	0.204	0.27	22	0.7	0.4612
Ring year 2012	−0.338	0.29	371	−1.2	0.2495
Ring year 2013	0.666	0.25	371	2.7	0.0079
Ring year 2014	0.504	0.16	371	3.1	0.0022
Ring year 2015	0.634	0.13	371	4.8	<0.001
Ring year 2016	0.405	0.12	371	3.4	<0.001
Ring year 2017	0.602	0.12	371	5.1	<0.001
Ring year 2018	0.318	0.12	371	2.7	0.0075
Aspect south	0.866	0.18	103	4.7	<0.001
Aspect west	0.639	0.24	103	2.7	0.0082
Aspect north	0.593	0.19	103	3.1	0.0025
Aspect east	0.503	0.24	103	2.1	0.037

All tree rings, except for year 2012, had significantly increased $\delta^{13}C$ values compared to pre-treatment rings (Table 3). This was particularly true for 2013, 2015 and 2017. The Snežnik study site had significantly increased average $\delta^{13}C$ values compared to the Rog site (Table 3, Fig. 3), whereas Trnovo site did not differ significantly from other two sites.

In case of aspect, all four categories (south, west, north, east) had significantly higher $\delta^{13}C$ values than the central position (bottom of the sinkhole with flat terrain). This difference was the highest (0.87‰) for south-facing microsites (Table 3), indicating that beech trees in canopy gaps growing on south-facing slopes had on average the highest $\delta^{13}C$ values (Fig. 4).

The microclimatic parameters T_{max}, RH_{min} and VPD_{max} differed significantly among different cutting intensities and also within-sinkhole positions (Table 4). The south-facing slopes were characterized by the most stressful conditions for plant growth, as manifested by the highest T_{max} and lowest RH_{min}, resulting in high VPD_{max} values. In contrast, the north-facing slopes (positioned in southern parts of the sinkholes) had experienced less extreme microclimate. A significant positive relationship was found between VPD_{max} and $\delta^{13}C$ values (Fig. 5).

Although soil conditions were less important, we also examined patterns in $\delta^{13}C$ values in relation to soil depth. In the initial global model with all five predictors, the model estimate (deep soil category as the reference) for intermediate soil was 0.147 ($p = 0.435$) and model coefficient for shallow soil was 0.387 ($p = 0.022$). There was a tendency for shallow soils having increased $\delta^{13}C$ values compared to deep soils, a pattern that was evident for the 50% and 100% cutting intensities.

The relationship between $\delta^{13}C$ values and light conditions in the 50% cutting intensity in 2018 showed that beech trees growing in more open microsites with higher radiation levels exhibited significantly increased $\delta^{13}C$ values compared to trees growing in shadier microsites with lower light availability (Fig. 6).

3.2. Environmental predictors of oxygen isotope ratio

The minimum $\delta^{18}O$ value in our dataset was 15.3‰ and maximum reached 32.7‰. The final mixed-effect model for $\delta^{18}O$ (AIC_c = 2018.7) included two significant predictors: tree ring and soil depth (Table 5). By far the largest model estimate was detected for 2017 (1.80). Differences in comparison to the reference category (pre-treatment ring) were also significant for 2014, 2015 and 2018 (Fig. 7). Beech trees growing on intermediate and more shallow soils had significantly higher $\delta^{18}O$ values compared to saplings on deep soil (Table 5, Fig. 8).

We found a marginally significant relationship between precipitation

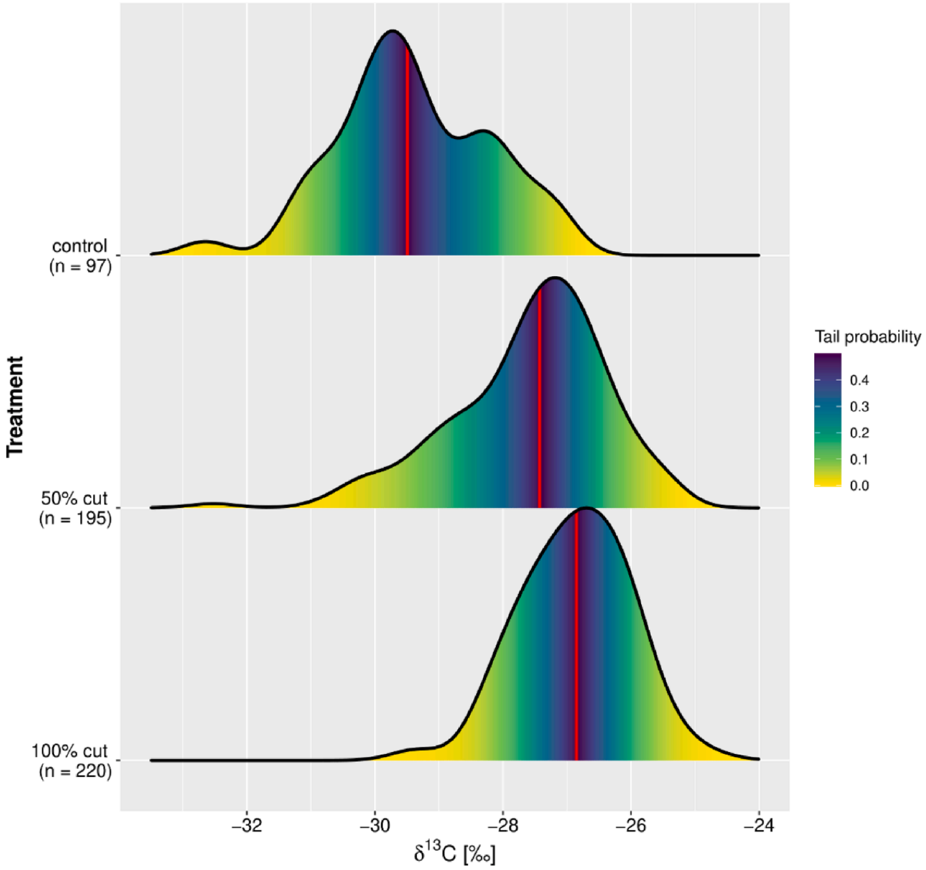

Fig. 2. Density plots of the $\delta^{13}C$ values for different cutting intensities (control, 50% cut, 100% cut). Red vertical line shows the median; n – number of samples (512 in total, from 135 sampled beech saplings).

amount in summer and $\delta^{18}O$ values (Fig. 9). The linear model indicated a decreasing trend of $\delta^{18}O$ along the summer precipitation gradient, suggesting that the climatic signal was rather weak. Note that an observation with high $\delta^{18}O$ (~ 28‰) reflects extremely dry and hot summer 2013 at the Snežnik site.

4. Discussion

Stable isotope-based information on the physiological performance of young trees studied in beech-dominated forests in the Dinaric Mountains (Slovenia) provides valuable insight into the mechanisms driving regeneration dynamics (Powers et al., 2011). Responses of natural regeneration to silvicultural interventions should represent a frequent example of stable isotope analysis because knowledge about factors affecting post-disturbance development in the early stages of succession is crucial for forest management. Due to climate change and large-scale forest disturbances, patterns of tree layer regeneration are predicted to alter and some key mesic species with moderate requirements for moisture (e.g., *Fagus sylvatica*) may not be able to cope with the foreseen increases in temperature and vapour pressure deficit (Bilela et al., 2012; Vitali et al., 2021).

Building upon theoretical assumptions underlying the interpretation of stable isotope signatures, we have shown that stable isotope composition in tree rings of beech saplings is associated with spatio-temporal environmental variations induced by silvicultural treatments and microsite factors. The main advantage of our research is the implementation of dual isotope approach (Roden and Farquhar, 2012; Giugiola et al., 2016). More detailed physiological conclusions can be drawn when $\delta^{13}C$ values are combined with $\delta^{18}O$ from the same samples (Cernusak and Ubierna, 2022). Secondly, because several external factors affect isotope composition simultaneously (Saurer et al., 1997; Geßler et al., 2001; Keitel et al., 2006), we examined the variation in stable isotope composition with respect to both macroclimates, operating at larger spatial scale and independently of forest management, and local effects (microclimate, soil properties), which depend on disturbance intensity or are modulated by microsite factors.

4.1. *Effects of forest management on tree-ring isotopes*

The most striking pattern in our dataset was the increase in $\delta^{13}C$ along the gradient of cutting intensity. This confirms strong differences in physiological and environmental determinants of stable isotope composition among applied silvicultural treatments. While overstory canopy reductions leads to more favourable light conditions in the forest understory, regenerating trees experience significant changes in microclimate. Such changes were found in the $\delta^{13}C$ signatures of young beech trees. With the increasing cutting intensity, tree-rings of beech saplings were less ^{13}C-depleted. On average, $\delta^{13}C$ values in trees from canopy gaps were nearly 2‰ increased (less negative) than in tree rings from closed stands (control). This is consistent with the results of Fotelli et al. (2003) demonstrating ^{13}C-enriched wood from beech seedlings in thinned stands compared to undisturbed control plots. Similarly, Powers et al. (2011) showed that seedlings of three pine species in harvested plots had greater $\delta^{13}C$, photosynthetic capacity and growth rate than seedlings in unharvested treatments.

The results from $\delta^{13}C$ measurements have been commonly used to interpret physiological changes in relation to A (photosynthetic assimilation rate) and g_s (stomatal conductance). Increased light availability and air temperature after 100% and 50% cutting intensity most likely triggered the increase in photosynthetic assimilation of young beech trees, thereby increasing the A/g_s ratio. Beech saplings in 50% cutting intensity experienced diverse light conditions, depending on their

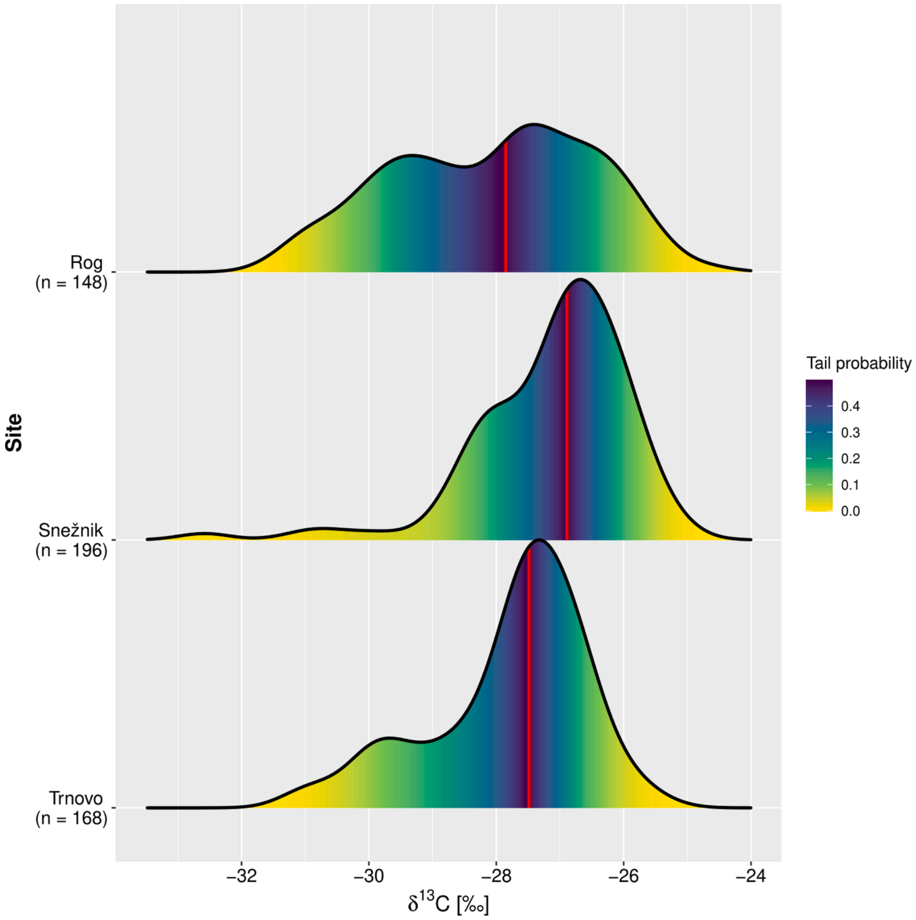

Fig. 3. Density plots of the $\delta^{13}C$ values for each study site (Rog, Snežnik and Trnovo). Red vertical line shows the median; n – number of samples (512 in total, from 135 sampled beech saplings).

position relative to the position of retained mature trees. Shaded juveniles showed decreased $\delta^{13}C$ values comparing to those in more open microsites with greater light availability. Decreased $\delta^{13}C$ values in tree rings are thought to reflect denser canopies being linked to increased shading (Mölder et al., 2011; Francey and Farquhar, 1982) and less stomatal limitation (Farquhar et al., 1982). Several studies revealed an increase in $\delta^{13}C$ with increasing irradiance (e.g., Hanba et al., 1997) and temperature (e.g., Dupouey et al., 1993). An increase in $\delta^{13}C$ could indicate stomatal closure and reduced conductance to prevent water loss during drought (Duquesnay et al., 1998; Keitel et al., 2006). Therefore, the elevated photosynthetic activity was likely constrained by decrease in stomatal conductance due to elevated VPD (combination of high temperature and low humidity). This parameter was on average 0.58 kPa (86%) higher in canopy gaps compared to closed stands. High sensitivity of beech $\delta^{13}C$ to VPD was shown by Vitali et al. (2021). Fotelli et al. (2009) documented that patterns of $\delta^{13}C$ in the phloem were influenced by short-term changes in VPD, whereas $\delta^{18}O$ showed no significant variation between years and values were not indicative of plants suffering from drought stress. In our case, the importance of each physiological component to the $\delta^{13}C$ signature remains speculative as we did not measure A or g_s. It is also possible that the increase in $\delta^{13}C$ was caused solely by increased photosynthetic capacity following management disturbance. This would support the hypothesis that $\delta^{13}C$ increased due to the higher increase in A over g_s, which was observed at forest sites where water is not a limiting factor (Di Matteo et al., 2017). Nevertheless, differential microsite conditions potentially influenced carbon isotope discrimination through soil depth and its effects on water status.

Management-induced changes in environmental factors acted as determinants of the carbon isotope composition. Controlling variables differed between carbon and oxygen isotope ratios. In general, $\delta^{13}C$ was much more sensitive to microclimate than $\delta^{18}O$. Changes in microclimate are likely the main drivers in $\delta^{13}C$ increases with a decrease in relative humidity and an increase in leaf temperature associated with tree cutting (Brooks and Mitchell, 2011). Despite profound differences in relative humidity between uncut reference stands and canopy gaps, cutting intensity was not an important factor in $\delta^{18}O$. This finding supports the results of Powers et al. (2011). According to their interpretation, the unresponsiveness of oxygen isotopes to thinning may indicate that the observed difference in $\delta^{13}C$ between control stands and cutting treatments is mainly due to higher photosynthetic rates rather than differences in g_s. On the other hand, non-significant increases in $\delta^{18}O$ along the cutting intensity gradient suggest greater g_s in treated plots than in controls, coupled with either a corresponding increase in A_{max} or evaporative demand (Powers et al., 2011). However, the difficulty in interpreting the results lies in the interplay of various, sometimes counteracting effects of variables (water, light) along environmental gradients (Keitel et al., 2006).

4.2. *The role of factors not directly linked to silviculture*

Given the multifactorial nature of the isotope-environment relationship, various factors control isotopic composition variability under most conditions, hence correlations with a single environmental parameter are simplifications. The relatively large variation in $\delta^{13}C$ values within each treatment suggests that carbon isotope composition may additionally depend not only to the effects of silvicultural treatments, but also on factors that act independently from management.

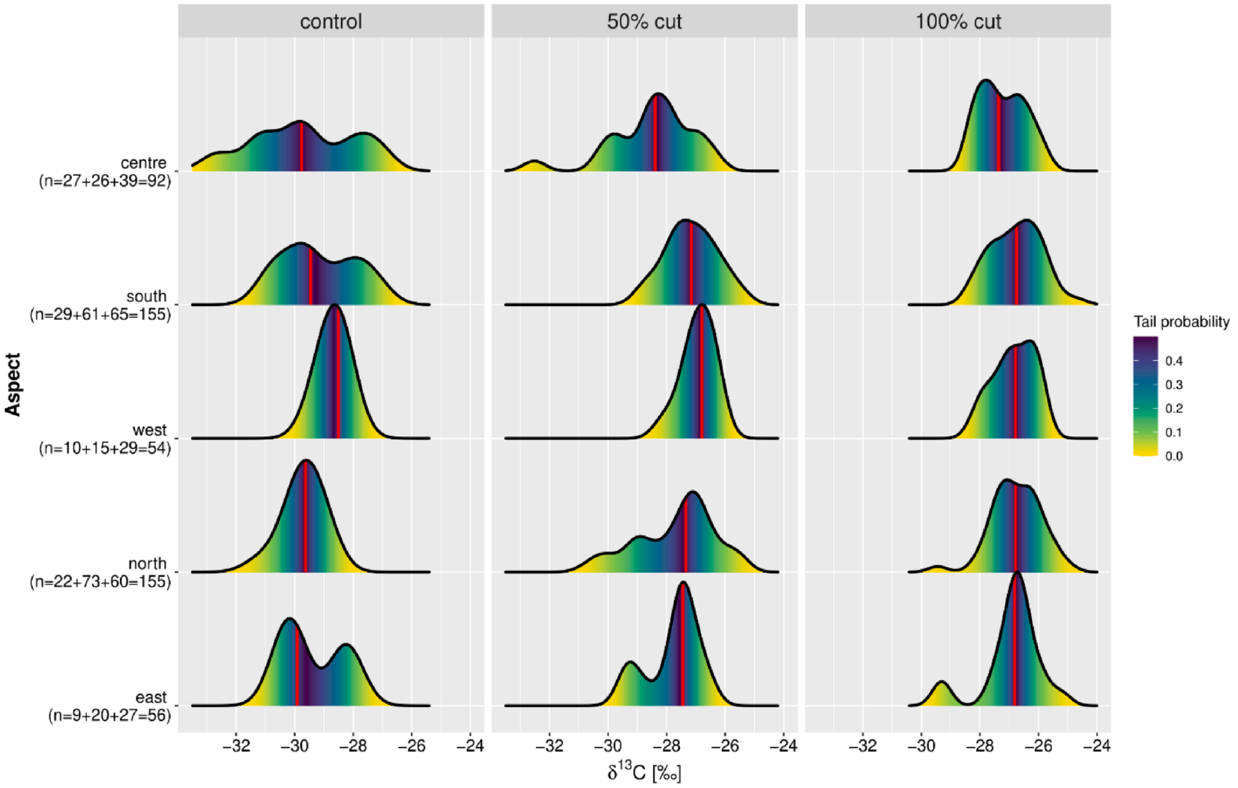

Fig. 4. Density plots of the $\delta^{13}C$ values for different slope aspects (centre, south, west, north, east), separately for each cutting intensity. Red vertical line shows the median; n – number of samples (control + 50% cut + 100% cut; 512 in total, from 135 sampled beech saplings).

Table 4
Measured microclimatic parameters (maximum daily temperature (T_{max}), minimum daily relative humidity (RH_{min}), maximum daily vapor pressure deficit (VPD_{max})) for different cutting intensities and slope aspects. Microclimatic data was obtained with field measurements on 81 different microsites (see Kermavnar et al., 2020) and averaged across three growing seasons (May - October) 2012–2014.

	Central plots	North-facing plots	South-facing plots
		T_{max} [°C]	
Control	17.84	17.90	17.86
50% cut	19.78	19.31	20.26
100% cut	21.22	20.80	22.04
		RH_{min} [%]	
Control	73.27	72.58	71.93
50% cut	65.01	66.06	63.95
100% cut	59.59	60.78	58.34
		VPD_{max} [Pa]	
Control	667.25	670.97	679.48
50% cut	982.58	921.91	1057.37
100% cut	1236.61	1170.76	1342.68

Microsite factors not directly linked to silvicultural effects may contribute significantly to the balance between photosynthetic carbon gain and water loss through transpiration when environmental conditions change. In our case, two factors that showed prominent fine-scale variation are slope aspect and soil depth. Both varied because the sampled beech trees grew in different positions within karst sinkholes. Slope aspect had a significant effect on microclimate, an important determinant of isotope composition. The variation in $\delta^{13}C$ between aspects can be explained mainly by differences in microclimatic conditions.

Microclimatic gradients in Dinaric fir-beech forests are controlled by the interaction between management intensity and local topography (Kermavnar et al., 2020). Higher VPD (a combination of high temperature and low relative humidity) in disturbed plots implies higher evaporative demand and under such conditions plants react by closing stomata to maintain leaf water potential. We found that saplings located on south-facing slopes had on average 0.42‰ higher $\delta^{13}C$ values in comparison to those on north-facing slopes. Fotelli et al. (2003) reported that the wood of beech seedling was ^{13}C-enriched on the SW compared to the NE aspect. This difference was attributed to differential light regime and associated transpiration rates and plant water potential in young trees, with a south-facing site receiving more radiation than a north-facing stand. Microsite exposition was identified as a driving factor for absolute $\delta^{13}C$ levels in the study of Treydte et al. (2001),

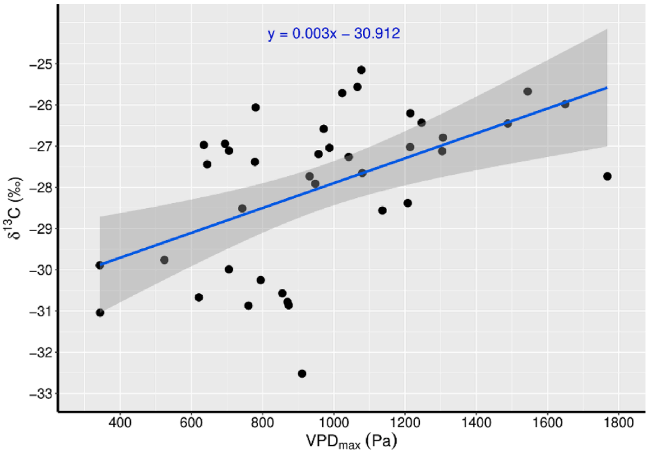

Fig. 5. The results of regression analysis between $\delta^{13}C$ and maximum daily vapor pressure deficit (VPD_{max}). Grey band around the trend line is the 95% confidence level interval for predictions from a linear model. Model statistics: F = 13.63, $p < 0.001$, $R^2 = 0.27$, n = 39.

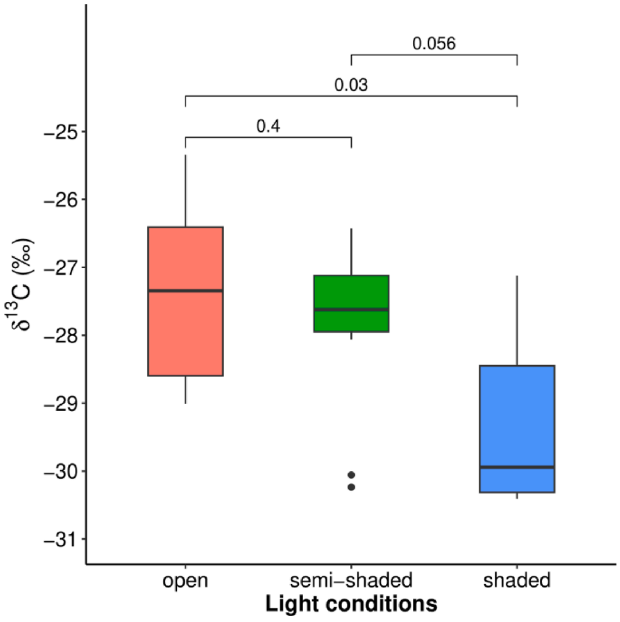

Fig. 6. The $\delta^{13}C$ values in 50% cutting intensity across three categories describing microsite-level light conditions: open (tree layer cover < 33%), semi-shaded (tree layer cover between 33% and 67%) and shaded (tree layer cover > 67%). According to Kruskal-Wallis test, p-values for pairwise comparisons are reported above boxplots: differences are significant for “open” vs. “shaded” comparison ($p < 0.05$).

Table 5
Fixed effects of the two categorical predictors (tree-ring and soil depth) for $\delta^{18}O$. Pre-treatment tree-ring block for year and deep soil for depth were used as reference categories for coefficient estimates. Data analysis was based on 512 samples (tree rings) extracted from 135 sampled beech juveniles.

	Estimate	Std. error	DF	t-value	p-value
Intercept	22.880	0.23	371	99.4	<0.001
Ring year 2012	0.863	0.56	371	1.5	0.1238
Ring year 2013	0.714	0.47	371	1.5	0.1335
Ring year 2014	0.671	0.31	371	2.2	0.0305
Ring year 2015	0.770	0.25	371	3.1	0.002
Ring year 2016	0.389	0.23	371	1.7	0.0877
Ring year 2017	1.804	0.22	371	8.1	<0.001
Ring year 2018	0.485	0.22	371	2.2	0.0297
Soil intermediate	0.723	0.29	105	2.5	0.0134
Soil shallow	0.754	0.24	105	3.1	0.0024

reporting that south exposures exhibit higher values than north exposure. Microclimatic differences between forest (micro)sites should be reflected in evaporation rates and hence available soil water, with south-facing sites experiencing greater summer water deficit than north -facing sites (Geßler et al., 2001).

Edaphic conditions in Dinaric fir-beech forests are highly variable and thus represent an important microsite factor acting at a small spatial scale. Mostly, the soils are shallow, averaging <20-cm depth of topsoil before being dominated by parent rock, interspersed with pockets of organic matter and mineral soil (Hukić et al., 2021). Soil depth (a proxy for soil moisture) proved to be a significant predictor of $\delta^{18}O$ but not $\delta^{13}C$, despite the significant correlation between the response variables in our dataset (Pearson correlation coefficient: 0.35, $p < 0.001$). There was a trend for $\delta^{13}C$ values to be increased on shallow soils, possibly indicating a more favourable water supply for beech saplings on microsites with deeper soil. Potential water deficiency on shallow soils should cause stomatal closure, an increase in the A/g_s ratio and consequently lead to increased $\delta^{13}C$ (Lauteri et al., 1997; Fotelli et al., 2003). Based on the strong correlations between $\delta^{13}C$ and soil water potential reported for beech (Fotelli et al., 2001), we assume that differences in the water status of young trees between soil depth categories may be responsible for the increased stomatal conductance due to enhanced water availability and observed patterns in $\delta^{13}C$ (Dawson and Siegwolf, 2007). Increases in $\delta^{18}O$ in cellulose indicate reduced root water uptake (Battipaglia et al., 2008). Although deep soil is presumed to be more favourable for tree growth in terms of water availability, deeper soils often have higher soil clay content. In such a soil texture, the plant-available water may be low despite a better water storage capacity because water is stuck in the fine pores (Kermavnar et al., 2023).

In interpreting the patterns in carbon isotope composition, some additional specificities associated with our study plots should be also considered. For instance, the southern parts of studied karst sinkholes were often characterized by steeper, rocky terrain with shallow soils, but at the same time less exposed to microclimatic extremes (lower VPD). In contrast, the northern parts were characterized by deeper soils and rather gentle topography. This could lead to a mixing effect of two factors, i.e., slope aspect and soil depth. Therefore, the differences in $\delta^{13}C$ within the karst sinkholes would likely be even more expressed if more sun-exposed microsites on northern parts would also have shallow soils.

In addition, observed changes in $\delta^{13}C$ over time could be in part due to potential juvenile effects. Wood formed during the first years of tree’s life is generally depleted in ^{13}C in comparison with wood formed later (Francey and Farquhar, 1982; Saurer et al., 1997). Juvenile effect has been reported in the literature but with high variability in strength and time span of the increase in $\delta^{13}C$ (Duquesnay et al., 1998). Such age-related trends could cause year-to-year changes (from the pre-treatment rings to the years between 2012 and 2018) in $\delta^{13}C$ values and thus might exert confounding patterns which cannot be directly attributed to management effects or alterations in light and water availability.

4.3. Large canopy gaps as stressful environments for beech regeneration

The increase availability of resources (light, temperature) associated with forest disturbances are beneficial for plant photosynthetic rate. This is the reason why seedlings and saplings of tree species in thinned stands and canopy gaps exhibit faster growth and greater height and radial increments. However, this is only one part of the equation describing a balance between carbon gain and water loss. Photosynthesis is maintained at a high level as long as humidity and water supply are sufficient. Canopy openings reduce the microclimatic buffering effect of forests. Owing to increased temperature, relative humidity is reduced in canopy gaps and thinned stands as shown by microclimatic measurements in our plots. This results in substantially elevated VPD in such environments. The rise in temperature exposes plants to high levels of evaporative demand, which leads to stomatal closure (Grossiord et al., 2020). Increased photosynthetic limitations arising from decreased stomatal conductance at high VPD pose constraints on tree growth and regeneration (Mölder et al., 2011). Thom et al. (2022) concluded that the positive effect of increased light availability on tree regeneration in European beech forests might be offset by harsher microclimate (maximum VPD) after canopy disturbances. Such negative effects on plant performance are even amplified during periods of water shortage. Both atmospheric aridity and drought influence the physiological function of plant leaves (Lavergne et al., 2020). In general, increased $\delta^{13}C$ values indicate more stressful environmental conditions, and $\delta^{13}C$ variations in tree rings of beech have been reported to indicate drought signals during the main growing season (Skomarkova et al., 2006).

The environment in canopy gaps is generally not uniform but shows profound variation in resource availability. This microsite heterogeneity is reflected in the uneven distribution of tree species regeneration layer (Vilhar et al., 2015) and herbaceous vegetation (Fahey and Puettmann, 2007; Kermavnar et al., 2019b) in gaps, which is known as the gap partitioning phenomenon. Silvicultural investigations imply that beech

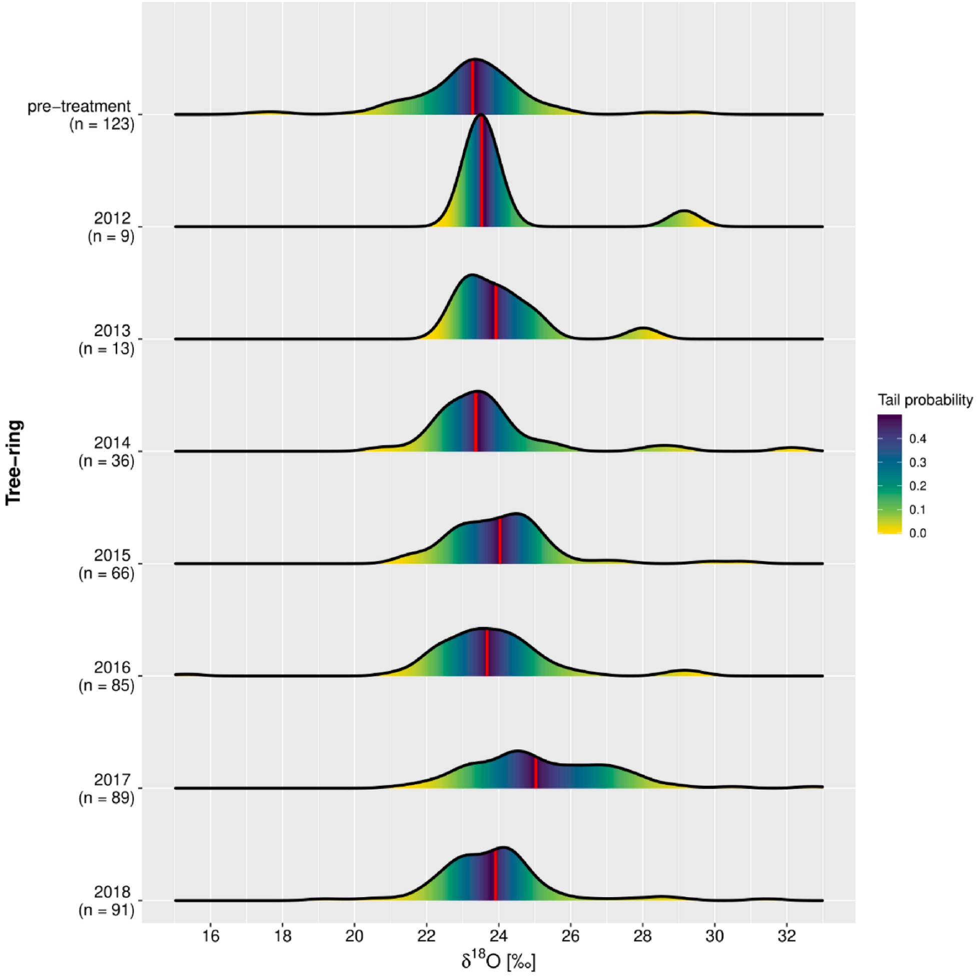

Fig. 7. Density plots of δ^{18}O values for different tree-rings from pre-treatment years (before 2012) to year 2018. Red vertical line shows the median; n – number of samples (512 in total, from 135 sampled beech saplings).

seedlings are more abundant on soils with higher moisture content and on concave microrelief (Diaci et al., 2020). Many species that form an advance regeneration seedling bank (e.g., *Fagus sylvatica*, *Abies alba*) are shade tolerant and show a negative relationship between direct light and regeneration success. An immediate large increase in direct light may inhibit their development or contribute to intensified post-disturbance mortality of mesic tree species, particularly at the northern gap edge (Bílek et al., 2014; Čater and Diaci, 2017). Differences in spatial patterns of understory vegetation within canopy gaps and partially harvested plots were identified by Kermavnar et al. (2019a), who concluded that microsites with more favourable microclimate (lower VPD) in southern parts (north exposure) of karst sinkholes contributed to persistence of typical forest plants while sun-exposed slopes (south exposure) were occupied by pioneer species with contrasting ecological niches.

From an eco-physiological point of view, our results clearly suggest that young beech trees growing in canopy gaps and thinned stands are experiencing stressful conditions. The stress is induced by higher temperatures and lower relative humidity, resulting in high VPD. These stressors are particularly strong in sun-exposed microsites (south-facing slopes) within karst sinkholes. In addition, we found that soil properties may also influence carbon and oxygen isotope composition. Shallow soils with presumably lower water availability compared to deeper soils were characterized by increased δ^{13}C and δ^{18}O values. Comparing two diametral situations (i.e., microsites with north exposure and deep soil in the control plots versus microsites with south exposure and shallow soil in plots with 100% cutting), the difference in δ^{13}C values was on average 3.4‰. The most stressful conditions for beech trees are likely to occur in open forest areas with hot and dry microclimate and shallow calcareous soils with low levels of soil moisture during summer when water demand is elevated. In addition, abiotic stress in disturbed areas may be amplified by intensified competition from ground vegetation or neighbourhood effects (Mölder et al., 2011). Dense ground vegetation can hinder the establishment of natural tree regeneration in the centre of gaps (Vilhar et al., 2015).

4.4. Study results in light of climate change

We found prominent year-to-year variations in δ^{13}C and δ^{18}O values in tree rings of beech saplings, which can probably be linked to inter-annual differences in mean and maximum temperatures, precipitation amount and associated humidity. The lowest mean δ^{13}C values in the 2012 tree rings could potentially indicate improved soil moisture availability immediately after tree cutting when herbaceous competition had not yet developed. Our 2012–2018 study period included growing seasons with contrasting meteorological conditions. For example, focusing on 2013 to 2015, we calculated that the summers were hotter (+3.1 °C and 3.7 °C, respectively) and drier (-51.3% and −15.6%, respectively) than the long-term average at all three study sites, i.e. for the reference period 1961–1990, which is usually used to detect climate anomalies by Slovenian Environment Agency (ARSO, 2022). However, summer 2014 was only + 0.8 °C warmer and received 15.6% more precipitation than the long-term average (Kermavnar et al., 2019c). Climatic differences between years manifested themselves to some degree in isotope composition. In the summer of 2014 (less hot and more

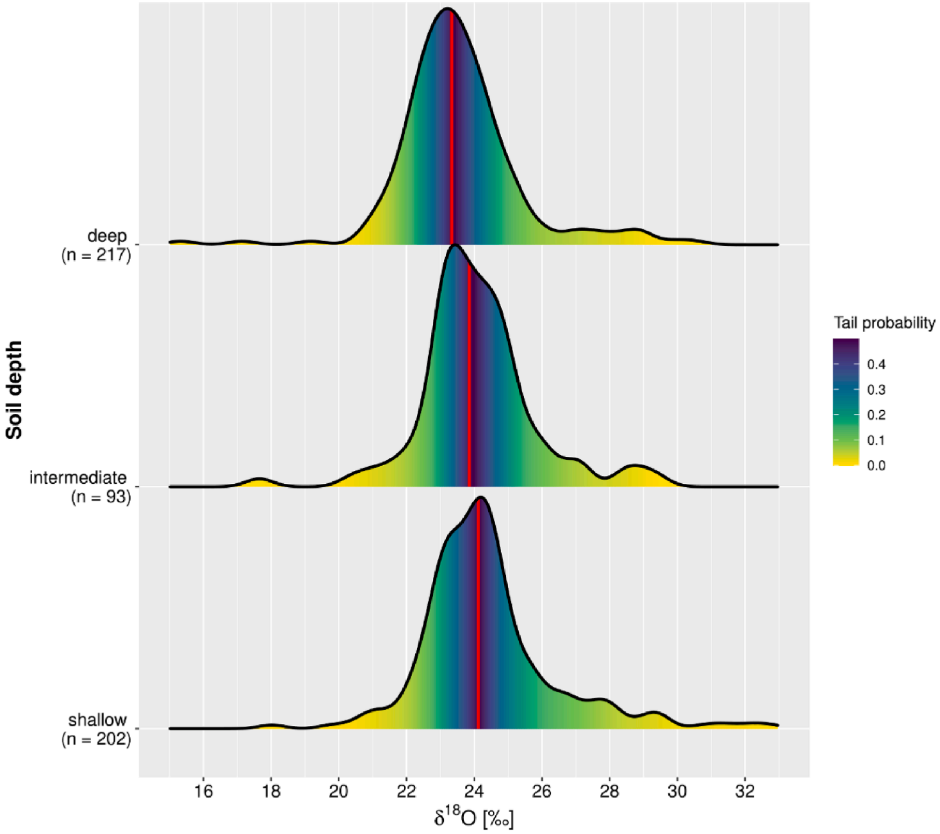

Fig. 8. Density plots of δ^{18}O values (‰) for three categories of soil depth (deep, intermediate, shallow). Red vertical line shows the median; n – number of samples (512 in total, from 135 sampled beech saplings).

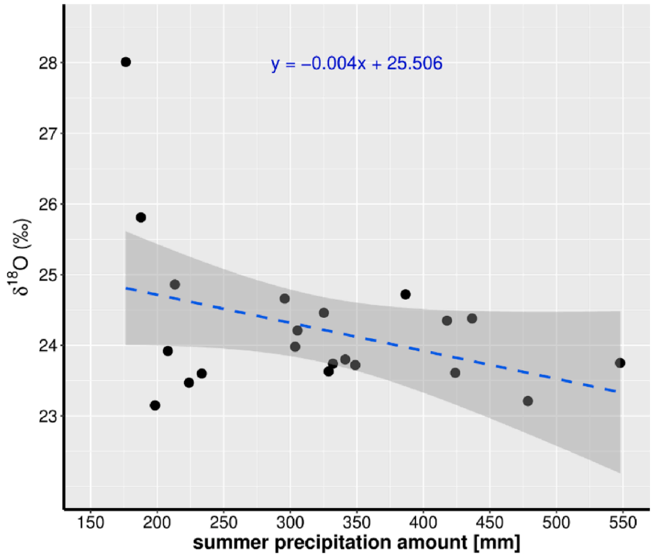

Fig. 9. The results of regression analysis between δ^{18}O and total summer precipitation amount. Grey band around the trend line is the 95% confidence level interval for predictions from a linear model (F = 3.26, $p = 0.09$, $R^2 = 0.15$, n = 21).

humid), the average δ^{18}O value was 23.8‰, whereas it was higher in the two hot and dry summers of 2013 and 2015 (24.1‰). Summer precipitation leads to higher air humidity and better water availability, both of which correlate negatively with oxygen isotope ratios in tree rings in various environments (Hilasvuori et al., 2009). For carbon isotopes, the differences were rather small, indicating a relatively weak climatic signal in our case. Saurer et al. (1995) concluded that δ^{13}C in beech tree rings was relatively high for dry/warm summers and low for cool/wet summers and that this relationship was significant only for dry sites but not for humid site. Potential explanation for the absence of macroclimatic signal in δ^{13}C may be the over-ridding effect of forest management (Skomarkova et al., 2006).

Situations of declining soil water supply and increasing atmospheric aridity are expected to become more frequent with climate change, particularly due to the combination of macroclimatic warming, compound weather events, and microclimatic extremes in open forest areas following large-scale forest disturbances (Gazol and Camarero, 2022). Heat waves are becoming more frequent, intense, and broader in spatial extent (Breshears et al., 2021). Hot summers tend also to be dry, so temperature correlates with relative humidity and soil moisture status, both of which directly affect stomatal conductance and therefore δ^{13}C. In the temperate forests of Central Europe, summer soil–water availability decreased over the past century (Saurer et al., 2014). The recurrent coincidence of high VPD and low soil moisture availability during summer may be the main trigger for mortality of forest trees (Timofeeva et al., 2017; Gazol and Camarero, 2022).

Drought-induced stomatal closure can significantly reduce carbon gain of temperate broad-leaved trees during drier summers even in the most humid climates of Europe (mountainous regions in the Alps, Carpathians and Dinarides). During severe summer drought, beech trees benefit from access to deeper and moister soils, allowing them to keep their stomata open. This mechanism is accompanied by increased water loss through transpiration, but at the same time allows for increased photosynthesis (Hartl-Meier et al., 2015). Intense drought events cause multiple negative consequences for beech trees and stands such as canopy defoliation, premature leaf senescence and growth reduction (Rohner et al., 2021; Gazol and Camarero, 2022). In Slovenia, defoliation of beech trees is increasing at an alarming rate (Ogris and Skudnik, 2021). Summer droughts can be even more detrimental to young trees than to adult trees as young trees do not have access to deeper soil layers due to shallow rooting of seedlings and saplings (Fotelli et al., 2003; Cernusak and English, 2015; Thom et al., 2022).

As the ongoing climate change continues, mesic forest types are likely to be under pressure due to the increasing effects of summer heat waves and co-occurring droughts, especially in area with low water storage capacity of the soil (Fotelli et al., 2003), such as many temperate forests in the Dinaric Mountains. The findings of García-Duro et al. (2021) suggested that climate change will alter forest composition and species abundance, with *Fagus sylvatica* forests being particularly vulnerable to climate change. Gazol and Camarero (2022) observed a steady increase in VPD in southern and eastern European regions, and forests in these regions are at high risk of increased tree mortality from drought. A dramatic decline in mesic beech forests to the benefit of more thermophilous forest vegetation types was projected for Slovenia (Kutnar and Kobler, 2011).

Although the studied sites in the north-western part of Dinaric beech forests distribution receive considerable amount of total annual precipitation (1500–2000 mm, which is above most sites in Central Europe), we have shown that summers are getting warmer and drier. Summer droughts are an important disturbance agent causing higher vulnerability of beech populations to biotic factors (pests and diseases) (Nagel et al., 2017), partly due to vicinity to the Mediterranean region. The Mediterranean and neighbouring temperate regions have been identified as evidently drought-prone biomes (Shestakova et al., 2019). The seasonal distribution of precipitation is shifting towards a Mediterranean-type climate, i.e. a pronounced precipitation deficit in the summer months. The rapid northward advance of Mediterranean climate caused by global climate change will affect demography of beech populations (Čater and Levanič, 2019). Dinaric fir-beech forests are especially exposed to climate change because of combined effects of unfavourable factors such as prolonged and reoccurring summer droughts, reduced mechanical and biological stability of forest stands due to large-scale disturbances, water-permeable karst terrain, and often shallow soils with low water storage capacity (Kermavnar et al., 2019c; Kutnar et al., 2021). Both mature and regenerating stands of beech grow on the shallow, rendzina soils derived from limestone and dolomite that are widespread in the Dinaric Mountains. Reductions in soil water availability will, arguably, strongly influence patterns of competition between drought-sensitive beech and tree species better adapted to water deficits.

5. Conclusions

The main goal of this study was to present the effects of management intensity on stable isotope composition in tree rings of beech juveniles. We additionally showed that within-sinkhole microsites differing in slope aspect and soil depth matter and may thereby act synergistically or antagonistically with the effects of tree cutting on beech regeneration patterns.

Some management implications can be derived from our findings. Thinned stands (50% cutting) and canopy gaps (100% cutting) had greater light availability, but significantly higher temperatures and lower relative humidity compared to undisturbed control plots. Harsh microclimate (high VPD) coupled with low levels of water content on shallow soils represent highly unfavourable conditions for establishment and growth of late-successional tree species in mesic beech-dominated forests pocked with karst terrain. Heat and drought extremes in the Dinaric Mountains are already forcing forest managers to find the right balance between increasing light availability and protecting regeneration from increasing atmospheric and / or edaphic aridity. Thus, in the context of climate change it is recommended that larger canopy openings should be avoided, and caution is needed even with respect to partial cuttings and commonly applied successive extension of gaps.

To minimize unwanted regeneration failures, the adaptation of cutting regimes to future climatic challenges should follow strategies that are in line with close-to-nature management practised in Slovenia (mimicking natural disturbance regime and adjusting planned interventions to local microsite conditions). The choice of optimal silvicultural method aimed at stimulating the natural tree regeneration with desired density and composition requires continuous monitoring of regeneration and vegetation dynamics over longer time periods, preferably supported with eco-physiological measurements. Stable isotope analysis goes beyond its application to tree regeneration, as it can be used for tracing the environmental stressors and signals of global change drivers that potentially threaten forest biodiversity.

CRediT authorship contribution statement

Janez Kermavnar: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Resources, Writing – original draft, Writing – review & editing. **Tom Levanič:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing. **Lado Kutnar:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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