



# Dissolved organic carbon leaching from montane grasslands under contrasting climate, soil and management conditions

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**Abstract** Grasslands are thought to be more vulnerable than many other ecosystems to climate change since their soils are characterized by high organic carbon contents and warming in montane regions is twice the global average rate. Despite these expected vulnerabilities, little is known about how climate change and management influence dissolved organic carbon (DOC) losses from montane grasslands and how relevant these losses are compared to other ecosystem carbon fluxes. In this study, 36 large (1 m<sup>2</sup>,

140 cm height) grassland lysimeters were filled with undisturbed soil monoliths and operated at three different sites located along an elevational gradient from 860 to 600 m a.s.l. From 2012 to 2014, changes in soil DOC concentrations and DOC leaching losses were quantified under different climate, soil and management (intensive vs. extensive; i.e., differing in the frequency of cutting and manuring events) conditions. The annual DOC leaching losses ranged between 6.6 and 27.5 kg C ha<sup>-1</sup> year<sup>-1</sup>, which was only a minor (< 3%) component of the net ecosystem carbon exchange. DOC leaching losses were not affected by management intensity but were positively correlated with soil organic carbon in the top soil. Climate warming (~ + 2 °C) significantly increased DOC leaching rates for intensive (+ 43%) and extensive management (+ 58%), but only if simultaneous reductions in leachate were moderate. The DOC concentrations in soil water significantly decreased with soil depth. In 10, 30 and 50 cm DOC concentration were positively correlated with aboveground biomass production, indicating that plants play a crucial role in topsoil DOC dynamics. However, this relationship did not occur for DOC in soil water collected at 140 cm, suggesting that adsorption and degradation processes in the subsoil ultimately determine the dynamics of DOC losses below the rooting zone.

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## Introduction

Grasslands are one of the most widely distributed land types in the world and cover approximately 30% of the Earth's land surface. In turn, grasslands are estimated to store approximately 20% of the world's carbon stocks (Schlesinger and Andrews 2000). Across Europe gross primary productivity (GPP) as well as net primary productivity (NPP) are substantially higher in grasslands than in forest and croplands, and taking into account other C gains (e.g. manuring) and losses (e.g. heterotrophic respiration, harvest) soils of grasslands were reported to be a significant C sink (Schulze et al. 2009). Overall, the aboveground and belowground C balance of grasslands highly depends on climatic and soil conditions and on grassland management such as fertilisation, cutting and grazing regimes (Soussana et al. 2007). Despite the important role of grasslands in European and even global carbon cycling (Parton et al. 1995), studies on DOC leaching have been mostly conducted in forest and peatland ecosystems (Kalbitz et al. 2000). Only few studies are available from grassland ecosystems (e.g., McTiernan et al. 2001; Chantigny 2003; Don and Schulze 2008; Hagedorn and Joos 2014) with montane grasslands being rather underrepresented.

Dissolved organic carbon (DOC) turnover and leaching can be an important component in terrestrial carbon (C) cycling. On the one hand, DOC leaching can provide a considerable pathway for ecosystem carbon losses (Siemens 2003; Kindler et al. 2011), and on the other hand, DOC can be sequestered in subsoils (Kaiser and Kalbitz 2012). Dissolved organic carbon is largely a decomposition product of litter and soil organic matter but it may also originate directly from root exudates. Though DOC accounts only for a small proportion of the total soil organic carbon (SOC), it plays also a significant role in influencing the dynamics of nutrient turnover such as denitrification (Bradley et al. 1992) as well as complexation and transport of contaminants (Sauvé et al. 2000) in soils. The production and consumption of DOC depends mainly on microbial activities and the equilibrium with the solid phase of soil organic matter (Chantigny 2003).

Thus, DOC production in soils is highly related to soil temperature, aeration, and pH conditions as well as management practices, such as liming and application of organic amendments and mineral fertilizer (Evans et al. 2002; Chantigny 2003; Van den Berg et al. 2012; Pisani et al. 2015). In addition to microbial processing, the fate of DOC strongly depends on soil water transportation (Tranvik and Jansson 2002), and subsequent adsorption/desorption is mainly influenced by Al/Fe oxides and clay minerals (Chantigny 2003; Kaiser and Kalbitz 2012). Due to the complex interaction of climate, soil, vegetation and management conditions, ecosystem DOC export was found to be site- and land-use dependent. Nevertheless, comparable rates of DOC losses ranging between 20 and 50 kg C ha<sup>-1</sup> year<sup>-1</sup> were reported for grassland and forest ecosystems (Kindler et al. 2011; Stergiadi et al. 2016; Manninen et al. 2018).

Due to the high stocks of soil organic carbon, particularly in mountain grasslands, these ecosystems are thought to be at risk of losing SOC via stimulated decomposition due to climate warming (Sjögersten et al. 2011; Wiesmeier et al. 2013; Puissant et al. 2017). This loss could be even more pronounced since temperature increases in European mountain regions are reported to be two times higher (Böhm et al. 2001) than the global average rate (Jones et al. 1999). Future changes in temperature will likely be accompanied by the alteration of precipitation regimes affecting both the intensity and seasonality (Gobiet et al. 2014). Shorter periods of snow cover, resulting in longer growing seasons, have been reported to coincide with substantial decline in summer precipitation and increasing drought risk (Smiatek et al. 2009), highly influencing vegetation dynamics (Jentsch 2013). Thus, changes in climate conditions affect carbon gains through net primary production (NPP) as well as C loss via respiration (autotroph and heterotroph) and DOC leaching, with the magnitude of the change ultimately determining the ecosystem carbon sink or source strength. However, the majority of climate manipulation studies in grasslands have focused on responses to plant productivity and CO<sub>2</sub> exchange, but only a few studies so far have investigated belowground carbon fluxes, such as DOC leaching (Hagedorn and Joos 2014; Puissant et al. 2017).

There is a larger body of work on how climate change affects DOC export from high latitude wetland and arctic permafrost ecosystems than for grasslands.

Freeman et al. (2001) have found a 65% increase (1988–2000) in the DOC concentration in freshwater drainage of peatlands in response to rising temperatures. Other work also showed that soils and peatlands under cold-climate conditions are particularly susceptible to temperature-driven increases in DOC production (Neff and Hooper 2002; Frey and Smith 2005; Dornblaser and Striegl 2015; Tank et al. 2016). However, as cause-effect chains are much more complex (Harrison et al. 2008) and high latitude permafrost soils and peatlands are characterized by different soil properties, thickness and hydrological patterns, it is questionable whether such trends can be extrapolated to other cold-climate systems such as grasslands at higher altitude, where such studies are less common.

In addition to climate change, different agricultural management practices may further affect DOC losses from soils. Over the past 60 years, management of grasslands has intensified, especially in Europe (Souchère et al. 2003). McTiernan et al. (2001) discovered that DOC loads from grasslands increased with increasing biomass production resulting from increasing rates of N fertilization (0–350 kg N ha<sup>-1</sup> year<sup>-1</sup>). Inputs of organic matter (e.g., by animal manure) can result in higher water-extractable organic carbon that likely increases potential DOC losses (Manninen et al. 2018). Bol et al. (1999) reported that organic fertilization significantly increased DOC leaching in grassland soils, which was either caused by a priming effect on DOC production from native soil C, or follows from dung-derived DOC, which partly displaced or substituted soil DOC adsorbed on the mineral phase of the soil. Management-induced changes in DOC generally have a short duration, whereas long-term effects are more related to the vegetation type and to the amount of plant litter returned to the soil. Due to the lack of studies and since many soil and plant properties are influenced simultaneously and can counterbalance each other the net effect of management practices on DOC dynamics still remains unclear (Chantigny 2003).

Therefore, a more thorough understanding how climate and land management influence grassland carbon exchange and budgets can be achieved only by more multifactorial field experiments considering different temperature, precipitation and agricultural land management regimes. Thus, the main aim of this study was to evaluate if DOC leaching is an important

component of montane grassland net ecosystem exchange and to clarify the effects of climate, soil and management conditions on DOC dynamics. We hypothesized that (i) DOC losses are higher in soils with high organic C contents, (ii) DOC losses are higher under higher management intensity (i.e. frequency of cutting and manuring events) and (iii) DOC losses will be increasing under climate warming. We evaluated these hypotheses with a long-term (3-year) continuous dataset (2012–2014) including measurements of DOC, plant and water dynamics at 36 large (1 m<sup>2</sup>, 140 cm height) lysimeters filled with undisturbed soil monoliths which were excavated from three different montane grassland sites.

## Materials and methods

### Site description

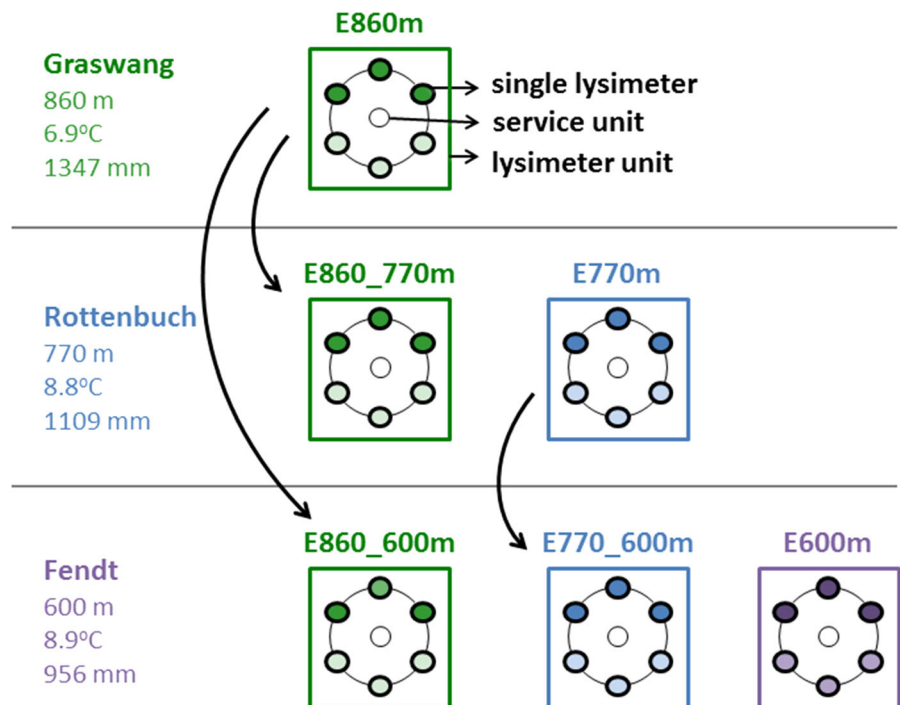
Investigations were conducted at three different grassland sites located along an elevation gradient in the TERENO Pre-Alpine Observatory (Kiese et al. 2018): E600m (Fendt: 47.82N, 11.06E, 600 m a.s.l.), E770m (Rottenbuch: 47.70N, 10.98E, 770 m a.s.l.) and E860m (Graswang: 46.56N, 11.03E, 860 m a.s.l.). Across the three sites with decreasing elevation, mean annual temperature (MAT) increases from 6.9 to 8.9 °C and mean annual precipitation (MAP) and leachate formation (PERC) decrease from 1347 to 956 mm year<sup>-1</sup> and 706 to 295 mm year<sup>-1</sup>, respectively. The soils at E860 are Cambisols characterized by higher top soil organic carbon (6.4%) and clay (52%) contents compared to the Stagnosols at E770m and E600m with SOC and clay contents of ~ 4% and 30%. The grassland vegetation consisted of grasses and herbs with minor (~ 10%) amounts of clover (*Trifolium repens*). The species communities at E860m are dominated by *Festuca pratensis*, *Poa pratensis*, *Prunella vulgaris*, *Plantago lanceolate*, *Knautia arvensis* and *Pimpinella major*, while species such as *Arrhenatherum elatius*, *Festuca rubra*, *Lolium perenne*, *Plantago lanceolate*, *Prunella vulgaris*, *Ranunculus repens* and *Taraxacum officinalis* are most common both at E770m and at E600m.

## Field set up and sampling design

From 2012 to 2014, DOC concentrations and leachate in 140 cm soil depth were measured from a total of 36 large 1 m<sup>2</sup> lysimeters that were filled with intact grassland soil monoliths. At any elevation (E600m, E770m, E860m), soil monoliths were excavated from three replicated sites and operated (N = 6) as a control at a central measuring location. Climate change conditions were simulated by the “space for time” concept, which anticipates climatic change through translocation of lysimeters in space, i.e. in our case from colder climates at higher elevations to warmer climates at lower elevations. To that end, six additional lysimeters from E860m each were translocated to E770m (i.e., E860\_770m) and E600m (i.e., E860\_600m) and six additional lysimeters from E770m were translocated to E600m (i.e., E770\_600m), resulting in 6, 12 and 18 lysimeters operated at E860m, E770m and E600m, respectively (Fig. 1). Translocation from E860m to E770m and E600m resulted in an increase in the mean annual temperature of 1.9 and 2.0 °C and reduction of annual rainfall by 18 and 29%, respectively (Table 1). Since there was hardly any temperature difference between E770m and E600m, only the translocated E860m soils

were included in the statistical analysis of the impacts of climate change. At each central measuring location, half of the lysimeters were subject to intensive management, and the other half were subject to extensive management. Extensive (low intensity) management includes up to three cuts and two manure applications, whereas intensive (high intensity) management is characterized by up to six cuts and five manure applications per year. These two management types are most common in the study region where harvests are generally used as forage for dairy farming. For grassland lysimeter management we followed the actual practices of local farmers in the years 2012 to 2014. The mean C and N loads per manure event are  $374 \pm 50$  kg C ha<sup>-1</sup> and  $42 \pm 10$  kg N ha<sup>-1</sup>, resulting in mean annual C and N additions via manuring of 748 and 1870 kg C ha<sup>-1</sup> and 84 and 210 kg N ha<sup>-1</sup> under extensive and intensive management, respectively. At each cutting event, plant biomass was manually harvested from each lysimeter, weighed after drying at 60 °C for 48 h (dry weight), and analysed for plant C (ISO 10694) contents (ISO 13878) at an external laboratory (Dr. Janssen GmbH, Gillersheim, Germany). At each elevation an eddy-covariance (EC) tower was operated for measurements of carbon exchange in the corresponding adjacent

**Fig. 1** Total of 36 lysimeters operated at three sites along an elevational gradient in the TERENO Pre-Alpine Observatory. E600m, E770m, E860m whereas E860\_770m, E860\_600m and E770\_600m represent climate change treatments i.e. E860m and E770m soils translocated (indicated by arrows) to lower elevations and warmer climates at E770m and E600m, respectively. Dark (intensive, N = 3) and light (extensive, N = 3) color codes represent differences in grassland management intensity



**Table 1** Climate (2012–2014) and soil characteristics (0–10 cm depth) of three montane grassland sites

Treatments	MAT (°C)	MAP (mm year <sup>-1</sup> )	leach_int (mm year <sup>-1</sup> )	leach_ext (mm year <sup>-1</sup> )	Elevation (m a.s.l.)	Soil type	Texture Sand/silt/clay (%)	Nt (%)	SOC (%)
Control	6.9	1347 ± 13	706 ± 37a	786 ± 58a	860	Fluvis Calcaric Cambisol	9/39/52 ± 2/1/3	0.7 ± 0.1	6.4 ± 0.6
E860m	8.8	1109 ± 16	570 ± 77b	585 ± 73b	770	Cambic Stagnosol	26/45/29 ± 1/1/2	0.5 ± 0.05	4.0 ± 0.3
E770m	8.9	956 ± 19	295 ± 40c	316 ± 40c	600	Cambic Stagnosol	27/43/30 ± 2/1/1	0.4 ± 0.04	3.9 ± 0.4
E600m									
Climate change	E860_770m Δ + 1.9 °C	Δ-238 mm	539 ± 45b	587 ± 41b	770				
	E860_600m Δ + 2.0 °C	Δ-391 mm	356 ± 30bc	352 ± 28bc	600				
	E770_600m Δ + 0.1 °C	Δ-153 mm	323 ± 31c	332 ± 26c	600				

Mean ± SE of annual temperature (MAT), precipitation (MAP) and leachate at 140 cm soil depth (leach\_int and leach\_ext for intensive and extensive managed lysimeters, respectively), total soil nitrogen content (Nt), SOC content. E860\_770m and E860\_600m stands for translocation of E860m grassland soils to E770m and E600m and E770\_600m for translocation of E770m grassland soil to E600m. Δ show differences of MAT and MAP to control conditions. Different lower case letters represent significant differences of leachate of intensive and extensive lysimeters between sites analyzed by multiple ANOVA statistics

grassland sites (Zeeman et al. 2017; Mauder et al. 2018).

Over the 3-year measuring period (2012–2014), soil water from 10, 30, 50 and 140 cm was continuously sampled from all lysimeters by vacuum (100 hPa representing mobile soil water) porous suction cups (SIC20&VS PRO, METER GROUP GmbH, Germany) connected to 1 L dark glass bottles mounted in their respective service units. Every second week, the glass bottles were emptied and a 50-ml sample of soil water was filtered through a 0.45 μm filter (Hydrophilic cellulose acetate membrane, Sartorius Stedim Biotech GmbH, Germany), frozen and stored for DOC and DON concentration analysis using a TOC/TN analyser (Multi N/C 3100, Jena Analytics, Germany). Annual DOC leaching rates (kg C ha<sup>-1</sup> year<sup>-1</sup>) were calculated by adding together leaching rates, calculated in biweekly time intervals from the product of DOC concentration (mg C l<sup>-1</sup>) and leachate volume (l) at the 140 cm soil depth. Leachate volume was derived from sub-daily weight changes in water tanks collecting excess soil water from the bottom of closed lysimeters via a suction rake connected to a pump, which was regulated according to in situ measured water tensions in 140 cm of undisturbed soil in close vicinity to the lysimeter station (for further details, see Fu et al. 2017). For better comparison of annual DOC leaching rates with other studies using a shallower sampling depth than 140 cm, we calculated annual DOC leaching rates also for 10, 30 and 50 cm soil depths. To obtain those rates, we multiplied the mean annual DOC concentration in a given soil layer with the annual leachate volume measured at the 140 cm soil depth, thus assuming that the same amount of water was percolating through any of the above soil layers. Comparing mean annual DOC leaching rates at 140 cm soil depth from this simple method (y) with the fortnightly calculated cumulative rates (x) revealed this method was highly robust ( $y = 1.06 \times x$ ;  $r^2 = 0.7$ ;  $p < 0.001$ ).

#### Data analysis

The effects of soil depth (10, 30, 50, 140 cm), management (intensive vs. extensive), growing (April–October) versus non-growing season (November–March), elevation (intensive and extensive controls at E860m, E770m, E600m), climate change



(control vs. translocated lysimeters of E860m) and soil type (soils of E860m, E770m and E600m operated under same climate conditions at E600m) were tested for DOC concentrations, DOC to DON ratio (DOC/DON), DOC leaching rates and aboveground biomass (AGB) production. Note that due to the violation of homogeneity of variances (revealed by Levene's test); all data were log-transformed before any analyses. Statistical tests for DOC concentration and DOC/DON were based on monthly mean values (2012–2014) and analysed by between-subjects effects of repeated measures ANOVA. If the ANOVA was significant ( $p < 0.05$ ), Tukey's HSD test ( $\alpha = 0.05$ ) was used for more detailed analysis of effects, e.g., across different soil depths, climate and soil treatments.

Statistical tests for DOC leaching and aboveground biomass were based on yearly values (2012–2014) and analysed by multiple (2-way, and 3-way depending on number of independent variables) ANOVA (Table 2). If the ANOVA was significant ( $p < 0.05$ ), Tukey's HSD test ( $\alpha = 0.05$ ) was used for a more detailed analysis of effects e.g., across different soil depths, climate and soil treatments.

For identification of the main environmental controls of DOC concentrations and DOC leaching rates, we used multiple stepwise regression as a forward procedure with MAT, MAP, C input via manure, AGB, SOC content in 10 cm (SOC10), and the mean annual DOC concentrations at any soil depth as predictor variables. All statistical analyses were performed with IBM SPSS Statistics 21.0 (IBM, Inc., USA) and Origin 9.0 (Origin Lab Corporation, USA).

## Results

### Variation of mean DOC concentrations

For all treatments and sites, the mean annual DOC concentrations in soil water significantly decreased with soil depth, and the concentrations ranged between 7.1 and 18.8 mg C l<sup>-1</sup> in 10 cm and 1.5–5.2 mg C l<sup>-1</sup> in 140 cm soil depths (Tables 2, 3). We found significantly higher DOC concentrations at all sites and treatments during the growing (April–October) season compared to the non-growing (November–March) season (Table 3; Fig. 2) with the overall highest DOC concentrations occurring in July

and August. Differences between the growing and non-growing seasons were most prominent at the 10 cm soil depth. These differences were highest at the lysimeters operated at E600m (E600m, E860\_600m and E770\_600m), the site characterized by the highest mean annual temperature (Table 1) and highest grassland biomass production (Fig. 3). DOC concentrations varied across elevation with significantly lower values at the E860m (Table 3), which is the site with the lowest mean annual temperature; however, this effect was only present in the topsoil (10 and 30 cm, Table 2). Comparing soils from different sites at same climatic conditions (Table 3) revealed highest concentrations at E600m (Table 2) characterized by lowest SOC content but highest plant productivity. Furthermore, DOC concentrations were significantly influenced by management and climate change conditions (Table 3). Mean DOC concentrations tended to be higher under intensive management with most pronounced differences in the top soil, but effects were only statistical significant at E860\_770m and E770m (Table 2). Although the experimental warming mostly increased mean DOC concentrations across all soil layers, significant differences occurred only at the 140 cm soil depth where we observed concentrations increases up to 3 mg C l<sup>-1</sup> (Table 2).

Considering all treatments and years, mean DOC concentrations in the top soil (10, 30 and 50 cm) were positively correlated with AGB production ( $\text{DOC}_{\text{conc}10} = 0.9 \times \text{AGB} + 1.3$ ,  $p < 0.001$ ;  $\text{DOC}_{\text{conc}30} = 0.5 \times \text{AGB} + 2.2$ ,  $p < 0.01$ ;  $\text{DOC}_{\text{conc}50} = 0.4 \times \text{AGB} + 1.3$ ,  $p < 0.01$ ), but not at the 140 cm soil depth. Other variables, such as C input via manure, SOC10, MAT and MAP were excluded since the increase in the prediction capacity of the regression was marginal and insignificant relative to AGB.

### Variation in the mean annual DOC to DON ratios

For all sites, treatments and soil depths, the mean DOC/DON in soil water ranged from 10.7 to 20.1 (Table 4), but in contrast to DOC concentrations, neither soil type and depth nor climate and management significantly influenced DOC/DON (Table 3). Nevertheless, the overall widest ratios were observed in the topsoil of E860m, which is the only site where DOC/DON significantly decreased with soil depth. Although climate change did not influence DOC/DON (Table 3), narrower ratios were found for E860\_770m

**Table 2** Mean  $\pm$  SE (2012–2014) DOC concentrations ( $\text{mg C l}^{-1}$ ) in 10, 30, 50 and 140 cm soil depth of intensive and extensive grassland lysimeters operated at three different sites along an elevational gradient

	Depth (cm)	E860m	E860_770m	E860_600m	E770m	E770_600m	E600m
Intensive management	10	10.3 $\pm$ 1.3 <sup>Ba</sup>	18.8 $\pm$ 3.9 <sup>a+</sup>	11.8 $\pm$ 0.8 <sup>a</sup>	18.8 $\pm$ 4.1 <sup>ABa+</sup>	13.2 $\pm$ 1.5 <sup>a</sup>	17.0 $\pm$ 0.7 <sup>Aa</sup>
	30	6.5 $\pm$ 1.3 <sup>Bb</sup>	10.4 $\pm$ 0.8 <sup>b</sup>	7.2 $\pm$ 0.6 <sup>b</sup>	9.9 $\pm$ 1.2 <sup>Bb+</sup>	6.7 $\pm$ 1.1 <sup>b</sup>	10.7 $\pm$ 1.1 <sup>Ab</sup>
	50	5.2 $\pm$ 0.9 <sup>Ab</sup>	8.9 $\pm$ 2.7 <sup>b+</sup>	5.7 $\pm$ 0.3 <sup>bc</sup>	8.5 $\pm$ 1.0 <sup>Ab+</sup>	5.1 $\pm$ 0.9 <sup>b</sup>	5.4 $\pm$ 0.6 <sup>Ac</sup>
	140	2.7 $\pm$ 0.6 <sup>Ab</sup>	3.7 $\pm$ 0.6 <sup>c*</sup>	3.7 $\pm$ 0.4 <sup>c*</sup>	3.7 $\pm$ 0.5 <sup>Ab</sup>	2.3 $\pm$ 0.4 <sup>c</sup>	2.6 $\pm$ 0.5 <sup>Ac</sup>
Extensive management	10	10.5 $\pm$ 1 <sup>Ba</sup>	8.6 $\pm$ 2.9 <sup>a</sup>	12.2 $\pm$ 1.3 <sup>a</sup>	7.1 $\pm$ 0.8 <sup>ABa</sup>	15.0 $\pm$ 2.6 <sup>a</sup>	17.5 $\pm$ 2.4 <sup>Aa</sup>
	30	5.5 $\pm$ 0.8 <sup>Bb</sup>	7.7 $\pm$ 3.5 <sup>ab</sup>	8.2 $\pm$ 1.7 <sup>b</sup>	6.4 $\pm$ 1.8 <sup>Bb</sup>	7.1 $\pm$ 1.6 <sup>b</sup>	11.0 $\pm$ 1.6 <sup>Ab</sup>
	50	3.7 $\pm$ 0.3 <sup>Bb</sup>	5.5 $\pm$ 0.8 <sup>b</sup>	6.0 $\pm$ 0.9 <sup>b</sup>	3.9 $\pm$ 0.8 <sup>Bb</sup>	5.2 $\pm$ 1.1 <sup>b</sup>	10.1 $\pm$ 1.3 <sup>Abc</sup>
	140	3.0 $\pm$ 0.4 <sup>Ab</sup>	5.2 $\pm$ 1.9 <sup>b*</sup>	3.8 $\pm$ 0.9 <sup>b</sup>	1.5 $\pm$ 0.2 <sup>Bb</sup>	4.3 $\pm$ 0.8 <sup>b</sup>	2.4 $\pm$ 0.3 <sup>ABc</sup>

Mean values were calculated from fortnightly measurements. Different lower case letters represent significant differences ( $p < 0.05$ ) across soil depths within one treatment and upper case letters across control sites (E860m, E770m, E600m). “+” represent significant differences of management at the same site while “\*” represent significant differences between control (E860m) and climate change (E860\_770m and E860\_600m) lysimeters. Statistically analyzes are based on repeated measures ANOVA followed by Tukey’s HSD

**Table 3** Effects (represented by F values) of soil depth, management, growing versus non-growing season, elevation, climate change and soil type on DOC concentrations, DOC to DON ratio, annual DOC leaching rates and above ground biomass production (AGB)

Lysimeters used for statistical analysis	Factors	DOC concentration	DOC/DON	DOC leaching rates	AGB
All (N = 36 lysimeters)	Soil depth	336.3***	0.5	n.a.	n.a.
	Management	8.8**	0.5	0.4	67.4***
	Grow-non-grow season	6.5*	0.03	4.8*	n.a.
Control lysimeters at any elevation: E860m, E770m, E600m (N = 3 $\times$ 6 = 18)	elevation	39.1***	2.7	7.3**	1.1
Control and translocated lysimeters of E860m: E860m, E860_770m, E860_600m (N = 3 $\times$ 6 = 18)	climate change	15.8***	0.4	3.5*	0.2
Lysimeters of any elevation operated at E600m: E860_600m, E770_600m, E600m (N = 3 $\times$ 6 = 18)	soil type	11.4***	0.6	3.8*	0.6

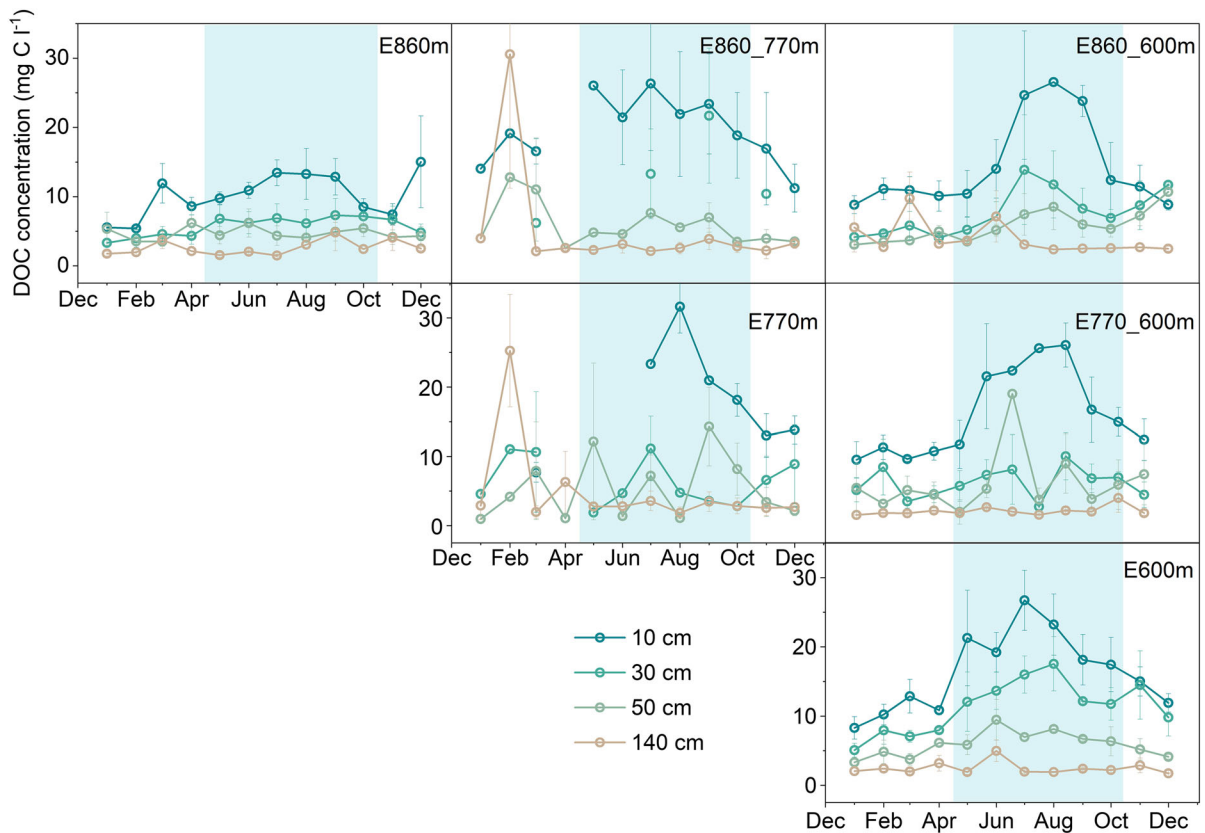
Note that statistics of concentration and ratio is based on monthly mean values of 2012–2014 and evaluated by between-subjects effects of repeated measures ANOVA, while statistics of DOC leaching rates was based on yearly values and evaluated by multiple ANOVA

Level of significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

as well as for E860\_600m at the 10 cm soil depth, both under intensive and extensive management conditions (Table 4).

Considering all treatments and years, mean DOC/DON were positively correlated with SOC10 but only

at the 30 and 140 cm soil depths ( $\text{DOC/DON}_{30\text{cm}} = \text{SOC10} \times 7.0 - 1.6$ ,  $p < 0.01$ ;  $\text{DOC/DON}_{140\text{cm}} = \text{SOC10} \times 12.5 + 6.1$ ,  $p < 0.05$ ). Other variables, such as C input via manure, MAT, MAP and AGB,



**Fig. 2** Course of monthly mean  $\pm$  SE DOC concentrations ( $\text{mg C l}^{-1} \pm \text{SE}$ ) of intensively managed grassland soils at three sites E860m, E770m and E770m along an elevational

gradient in the TERENO Pre-Alpine Observatory in 10, 20, 50 and 140 cm soil depth. Blue area represents growing season. (Color figure online)

were excluded since they did not further increase the prediction capacity of the regression.

eddy covariance towers at fields of soil excavation were  $198 \pm 48$ ,  $140 \pm 44$  and  $267 \pm 40 \text{ g C m}^{-2}$  for E860m, E770m and E600m, respectively.

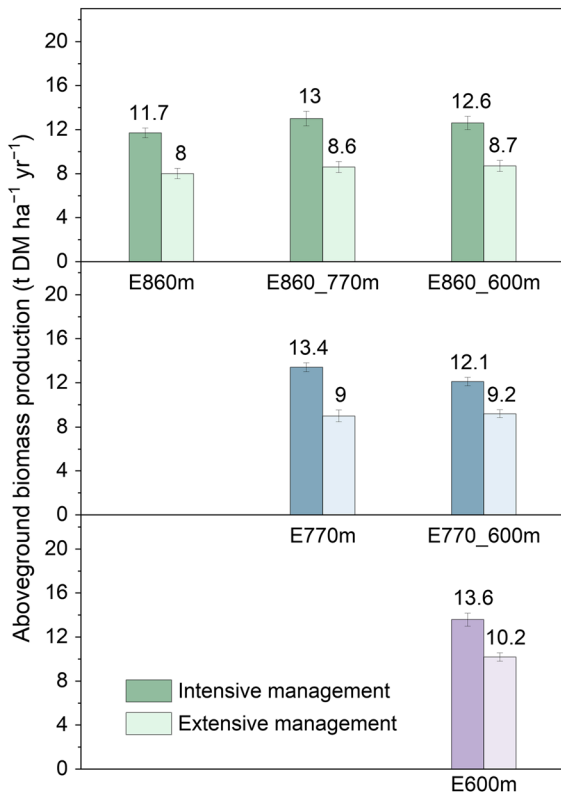
#### Variation of above ground biomass (AGB) production and NEE

#### Variation of mean annual DOC leaching rates

Across all sites and climate conditions, AGB production ranged between 11.7 and 13.6 t dry matter (DM)  $\text{ha}^{-1} \text{ year}^{-1}$  for intensive management, and thus, it was significantly higher than the range of 8.0–10.2 t DM  $\text{ha}^{-1} \text{ year}^{-1}$  observed for extensive management (Table 3; Fig. 3). Independent of management, AGB production at E860m was significantly lower than at E770m and E600m. Increasing AGB production under climate change conditions (i.e., at E860\_770m and E860\_600m) was only significant under intensive, but not under extensive, management (Fig. 3). As previously reported by Zeeman et al. (2017) mean annual (2012–2014) net ecosystem exchange measured by

Considering all treatments and sites, the mean (2012–2014) annual DOC leaching rates ranged from 6.6 to 27.5  $\text{kg C ha}^{-1} \text{ year}^{-1}$  but were not significantly different between intensive and extensive management (Table 5; Fig. 4). In comparing control grassland soils (i.e., E860m, E770m and E600m), the lowest leaching rates ( $< 10 \text{ kg C ha}^{-1} \text{ year}^{-1}$ ) were observed at the lowest elevation (E600m) while significantly higher values ( $> 17 \text{ kg C ha}^{-1} \text{ year}^{-1}$ ) were observed at higher elevations (except for E770m extensive, Fig. 4; Table 3). The significant trend (Table 3) in higher DOC leaching losses of E860m was also found when different soil types were





**Fig. 3** Mean ± SE (2012–2014) annual aboveground biomass production (t DM ha<sup>-1</sup> year<sup>-1</sup>) of intensively managed grassland soils at three sites E860m, E770m and E770m along an elevational gradient in the TERENO Pre-Alpine Observatory

compared under the same climate conditions (i.e., E660m, E770m\_600m vs. E860m\_600m). Annual DOC leaching rates were significantly altered under climate change conditions (Table 3). However, for both intensive and extensive management, stimulation of DOC leaching rates was only observed under moderately reduced (– 24%) leachate volume at E860\_770m but not at E860\_600 with a much higher (– 50%) reduction of leachate volume (Fig. 4; Table 1). For a better comparison of annual DOC leaching rates with other studies mostly using shallower sampling depths, we calculated the annual DOC leaching rates for the 10, 30 and 50 cm soil depths from mean annual DOC concentrations (Table 2) and assuming there was the same annual leachate volume as in 140 cm soil depth. According to decreasing DOC concentration with soil depth, Fig. 5 mirrors this decrease also with mean annual DOC leaching rates. Across all treatments, the average leaching rates in the 30 cm and 50 cm soil depths were a factor of 2.6 and 1.3 higher than the rates estimated for the 140 cm soil depth.

Multiple regression analyses revealed that the DOC concentration in the 140 cm soil depth alone explained 42% of the variance of annual DOC leaching losses ( $DOC_{leach} = 3.1 \times DOC_{conc140} + 4.6$ ,  $p < 0.001$ ) but topsoil DOC concentrations (i.e., 10, 30, 50 cm

**Table 4** Mean ± SE (2012–2014) DOC/DON in 10, 30, 50 and 140 cm soil depth of differently managed grassland lysimeters operated at three different sites along an elevational gradient

	Depth (cm)	E860m	E860_770m	E860_600m	E770m	E770_600m	E600m
Intensive	10	20.1 ± 2.9 <sup>aA</sup>	13.6 ± 0.6 <sup>b*</sup>	15.6 ± 1.4 <sup>*</sup>	12.9 ± 0.8	13.4 ± 0.8	15.1 ± 1.4 <sup>B</sup>
	30	19.2 ± 2.4 <sup>abA</sup>	15.3 ± 3.2 <sup>a</sup>	17.4 ± 1.4	16.4 ± 3.2 <sup>A</sup>	16.1 ± 1.7	13.5 ± 1.5 <sup>B</sup>
	50	16.6 ± 1.4 <sup>abA</sup>	15.9 ± 0.8 <sup>a</sup>	17.4 ± 1.5	12.7 ± 0.6 <sup>B+</sup>	19.3 ± 2.1	10.3 ± 1.1 <sup>B+</sup>
	140	14.6 ± 1.8 <sup>bA</sup>	13.3 ± 0.6 <sup>b</sup>	15.5 ± 1.2	14.6 ± 1.0 <sup>A</sup>	12.3 ± 1.3	10.7 ± 1.5 <sup>A</sup>
Extensive	10	18.2 ± 1.0 <sup>aA</sup>	16.3 ± 1.6 <sup>a*</sup>	14.6 ± 0.8 <sup>*</sup>	15.4 ± 0.8 <sup>B</sup>	15.1 ± 0.5	12.8 ± 0.7 <sup>C</sup>
	30	17.7 ± 1.3 <sup>a</sup>	21.8 ± 3.4 <sup>a</sup>	17.2 ± 1.4	16.5 ± 1.2	18.2 ± 1.4	14.8 ± 2.4
	50	15.7 ± 0.8 <sup>ab</sup>	13.9 ± 0.7 <sup>b</sup>	16.0 ± 0.7	16.6 ± 2.2	15.6 ± 0.8	15.8 ± 1.4
	140	14.5 ± 1.0 <sup>b</sup>	12.2 ± 0.8 <sup>b</sup>	16.1 ± 1.2	13.3 ± 1.0	15.5 ± 2.0	13.3 ± 0.8

Different lower case letters represent significant differences ( $p < 0.05$ ) across soil depths within one treatment and upper case letters across control sites (E860m, E770m, E600m). “+” represent significant differences of management at the same site while “\*” represent significant differences between control (E860m) and climate change (E860\_770m and E860\_600m) treatments. Statistically analyzes are based on repeated measures ANOVA followed by Tukey’s HSD

soil depth) remain unrelated to DOC losses at the 140 cm soil depth. The prediction capacity of the regression could be increased (57% of the variance explained) by further taking into account SOC contents in 10 cm soil depth, which were positively correlated with annual DOC leaching rates ( $\text{DOC}_{\text{leach}} = 3.1 \times \text{DOC}_{\text{conc140}} + 1.6 \times \text{SOC}_{10} - 4.0$ ,  $p < 0.001$ ). The C input via manure, MAT, MAP and AGB were excluded since they did not further increase the prediction capacity of the regression.

## Discussion

The annual DOC leaching rates observed for three montane grassland soils in this study ranged between 6.6 and 27.5 kg C ha<sup>-1</sup> year<sup>-1</sup>. Considering measurements from EC towers at corresponding adjacent grassland sites (Zeeman et al. 2017), the mean annual DOC losses reported for the years 2012–2014 were only 1.0 ± 0.3%, 2.0 ± 1.0% and 0.4 ± 0.1% of NEE at E860m, E700m and E600m, respectively. These ratios are at the lower end of reported values for similar systems and further support findings from earlier studies that DOC losses are only a minor

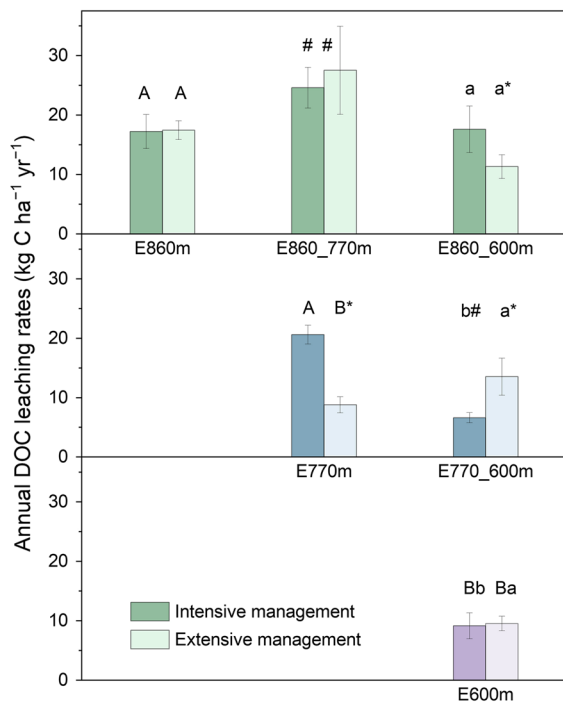
component determining grassland NEE (Kindler et al. 2011; Jones et al. 2017). Though DOC leaching losses increased by 43% (intensive) and 58% (extensive) under increasing temperatures, absolute increases are < 10 kg C ha<sup>-1</sup> (Table 5) and therefore, too low to significantly affect grassland C balances under climate change conditions. This is different to warming induced increases in AGB production ranging between 0.5 and 1.0 t C ha<sup>-1</sup> in 2012–2014 (Fig. 3). Overall, our DOC leaching rates are lower than the mean loss rates (53 ± 20 kg C ha<sup>-1</sup> year<sup>-1</sup>) reported for European grasslands in a review by Kindler et al. (2011), who used a comparable method of water sampling with suction cups and calculated leachate volume through a soil water modelling approach. However, their sampling depth was between 75 and 120 cm, which is much lower than ours at 140 cm. As previously demonstrated (Don and Schulze 2008; Sandermann et al. 2008; Kindler et al. 2011; Michalzik et al. 2001) and also supported by this study, DOC concentrations decrease with increasing soil depths (Table 2). Since our DOC concentration in the top soil (mean over all treatments in 10 cm: 13.4 ± 1.2 mg C l<sup>-1</sup>) compare well with other grassland data e.g. from the UK (15.4 ± 2.2 mg C l<sup>-1</sup>) and

**Table 5** Mean ± SE annual and yearly (2012–2014) DOC leaching losses (± SE) in kg C ha<sup>-1</sup> year<sup>-1</sup> of differently managed grassland lysimeters operated at three different sites along an elevational gradient as calculated by adding together

fortnightly DOC leaching rates, which were calculated from the product of DOC concentration and total leachate volume at the 140 cm soil depth

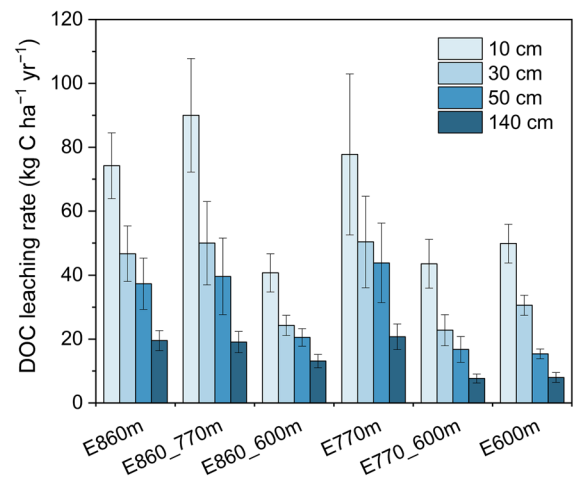
Treatments		Intensive	Extensive	Treatments		Intensive	Extensive
E860m	2012	15.2 ± 2.1	17.5 ± 1.0	E770m	2012	25.3 ± 5.8	9.7 ± 2.1
	2013	24.5 ± 6.7	22.3 ± 1.6		2013	16.3 ± 3.1	12.1 ± 1.6
	2014	11.9 ± 2.5	12.5 ± 1.3		2014	20.1 ± 6.0	4.6 ± 0.7
	<b>Avg.</b>	<b>17.2 ± 2.9<sup>A</sup></b>	<b>17.4 ± 1.6<sup>A</sup></b>		<b>Avg.</b>	<b>20.6 ± 1.6<sup>A</sup></b>	<b>8.8 ± 1.4<sup>B+</sup></b>
E860_770m	2012	32.6 ± 2.6	44.4 ± 16.0	E770_600m	2012	6.8 ± 1.0	14.3 ± 3.3
	2013	17.0 ± 0.4	17.6 ± 4.8		2013	9.2 ± 0.5	21.0 ± 6.6
	2014	24.1 ± 6.9	20.5 ± 12.7		2014	3.8 ± 0.3	5.3 ± 1.0
	<b>Avg.</b>	<b>24.6 ± 3.4<sup>*</sup></b>	<b>27.5 ± 7.4<sup>*</sup></b>		<b>Avg.</b>	<b>6.6 ± 0.8<sup>b</sup></b>	<b>13.5 ± 3.1<sup>a+</sup></b>
E860_600m	2012	13.5 ± 2.6	9.8 ± 2.1	E600m	2012	7.4 ± 0.9	8.6 ± 0.4
	2013	27.7 ± 8.9	17.6 ± 4.5		2013	15.6 ± 4.7	12.2 ± 0.2
	2014	11.6 ± 4.6	6.6 ± 1.0		2014	4.4 ± 0.7	7.9 ± 3.5
	<b>Avg.</b>	<b>17.6 ± 3.9<sup>a</sup></b>	<b>11.3 ± 2.0<sup>a+</sup></b>		<b>Avg.</b>	<b>9.2 ± 2.2<sup>Bb</sup></b>	<b>9.6 ± 1.2<sup>Ba</sup></b>

Different upper case letters represent significant differences ( $p < 0.05$ ) across control sites (E860m, E770m, E600m) while different lower case letters represent significant differences across different soil types (E860\_600m, E770\_600m and E600m). “+” represent significant differences of management at the same site while “\*” represent significant differences between control (E860m) and climate change (E860\_770m and E860\_600m) treatments. Statistically analyzes are based on multiple ANOVA followed by Tukey’s HSD



**Fig. 4** Mean  $\pm$  SE (2012–2014) annual DOC leaching losses ( $\text{kg C ha}^{-1} \text{ year}^{-1}$ ) of differently managed grassland lysimeters operated at three different sites along an elevational gradient as calculated by adding together fortnightly DOC leaching rates, which were calculated from the product of DOC concentration and total leachate volume at the 140 cm soil depth. Different upper case letters represent significant differences ( $p < 0.05$ ) across control sites (E860m, E770m, E600m) while different lower case letters represent significant differences across different soil types (E860\_600m, E770\_600m and E600m). “\*” represent significant differences of management at the same site while “#” represent significant differences between control (E860m) and climate change (E860\_770m and E860\_600m) treatments. Statistical analyses are based on multiple ANOVA followed by Tukey’s HSD

elsewhere ( $14.9 \pm 5.0 \text{ mg C l}^{-1}$ ) (van den Berg et al. 2012), the lower annual DOC leaching rates can be partly explained by the different sampling depths. Indeed, our range of DOC leaching rates from 15 to  $44 \text{ kg C ha}^{-1} \text{ year}^{-1}$  estimated for a soil depth of 50 cm (Fig. 5), agrees much better with previously reported DOC leaching rates from grasslands. These findings highlight that comparing DOC leaching rates among studies reporting values for different sampling depths can be highly biased. Our lysimeter approach allows only vertical flow through the soil monoliths but cannot reflect lateral downslope movement of water and solutes at slopes. As we found decreasing



**Fig. 5** Estimates of mean  $\pm$  SE (2012–2014) annual DOC leaching rates ( $\text{kg C ha}^{-1} \text{ year}^{-1} \pm \text{SE}$ ) in 10, 30, 50, 140 cm of intensively managed grassland soils at three sites E860m, E770m and E770m along an elevational gradient in the TERENO Pre-Alpine Observatory as calculated from mean annual DOC concentration and annual leachate volume at 140 cm soil depth

DOC concentration with soil depth, our experimental setup would likely lead to overestimation of DOC leaching rates if lateral fluxes would be substantial. However, in our study region managed grasslands mainly occur at valley bottoms or on gentle slopes whereas steep slopes are covered by forests (Kiese et al. 2018). For that reason and because of low water conductivities of our soils characterized by high silt and clay contents (Table 1) it is unlikely that neglecting lateral fluxes will cause a large bias on our results. In a study of Fu et al. (2017) continuously low nitrate concentration in a stream at the Fendt site at E600m further support that fast lateral nutrient transfer from grasslands is likely of minor importance.

We found significantly ( $p < 0.05$ ) increased DOC losses only for lysimeters translocated from E860m to E770m (Fig. 4; Table 5), which represents an increase in MAT by  $1.9 \text{ }^\circ\text{C}$  and a reduction of MAP and leachate volume (intensive treatments) by  $-238$  and  $-167 \text{ mm year}^{-1}$ , respectively (Table 1). However, for translocated lysimeters from E860m to E600m ( $+2.0 \text{ }^\circ\text{C}$  MAT,  $-391 \text{ mm year}^{-1}$  MAP), no significant ( $p < 0.05$ ) difference in annual DOC leaching rates were observed for intensive nor extensive treatments. Since the mean DOC concentration in the 140 cm soil depth of E860\_600m also increased (Table 2), lower leaching rates are likely caused by

significantly lower leachate formation ( $-350 \text{ mm year}^{-1}$ ) at E600m compared to E770m. This result demonstrates that climate change-induced changes in DOC leaching rates are highly dependent on changes in soil water fluxes. These changes can even overwrite the stimulating effects of temperature on increasing DOC concentration, particularly if they are small ( $+0.8$  to  $2.2 \text{ mg C l}^{-1}$ ), as observed for the 140 cm soil depth (Table 2). This is in line with findings of earlier studies also showing that changes in precipitation and evapotranspiration can strongly influence leachate formation and thus the leaching rates of DOC (Tranvik and Jansson 2002; Don and Schulze 2008; Harrison et al. 2008; Hagedorn and Joos 2014). Overall increases of DOC leaching rates under moderate changes in leachate volume compare well with the findings of enhanced DOC leaching rates reported from other grassland studies (Puissant et al. 2017) as well as a meta-analysis considering different terrestrial ecosystems by Lu et al. (2013). As our results show, DOC leaching is controlled by both soil DOC concentration and soil water dynamics, which can vary highly across ecosystems varying in chemical and physical soil properties and soil thickness. Due to overall highest DOC concentrations in the top soil, shallower soils above bedrock or permafrost blockage might be more vulnerable to increased DOC export than deeply weathered soils (Dornblaser and Striegl 2015), as those found in our study region, likely characterized by a higher retention capacity or microbial degradation of DOC in the subsoil (Kalbitz et al. 2000). To what degree significant lower soil water DOC concentration in sub soils (140 cm) are caused by microbial metabolism or physical adsorption remains unclear. The latter is supported by high clay contents of the investigated soils (Table 1), but substantial reductions in soil  $\text{NO}_3$  concentration from 50 to 140 cm soil depth and substantial fluxes of  $\text{N}_2$  as reported for the same soils by Fu et al. (2017) and Zistl-Schlingmann et al. (2019) respectively, might indicate DOC metabolism by microbial denitrification.

In our study, aboveground biomass production was positively correlated with the DOC concentrations in the upper 50 cm of the soil, indicating that plants play a crucial role in topsoil DOC dynamics via litter production and root exudation (Kalbitz et al. 2000; McTiernan et al. 2001). The importance of fresh organic matter inputs triggered by plants is further supported by the seasonal variability of the DOC

concentrations with higher concentrations during the growing compared to the non-growing seasons with the most pronounced differences occurring in the main rooting zone (0–10 cm) (Fig. 2). Similar seasonal patterns were reported by other studies (Guggenberger and Zech 1994; Scott et al. 1998; van den Berg et al. 2012) but it is likely that seasonality of air and soil temperature was also influencing temporal variability of DOC concentration. Van den Berg et al. (2012) could not relate temporal variability of DOC concentration to changes in air temperature, which agrees with our findings and with Michalzik et al. (2001) for temperate forest ecosystems. These results indicate that on annual scale and across sites DOC concentrations and leaching rates were not correlated with MAT, which is relativizing the importance solely of temperature on DOC dynamics. The correlation of DOC concentrations and AGB was not present for the 140 cm soil depth, which likely occurred due to subsoil DOC adsorption and microbial degradation further influencing DOC concentration and thus leaching dynamics (Kalbitz et al. 2000). However, to elucidate the importance and interrelation of subsoil processes, further studies should consider the use of stable isotope techniques and detailed chemical and structural characterization of DOC which have been successfully applied to elucidate sources and sinks of DOC as water percolates through the soil profile (Hagedorn et al. 2004; Sandermann et al. 2008; Wang et al. 2019).

Comparing different soils under the same climatic conditions revealed that subsoil DOC concentrations and leaching losses of soils from E860m transferred to E770m (E860\_770m) and E600m (E860\_600m) were significantly higher than the controls (E770m and E600m), no matter which management regime was used (Fig. 4; Tables 2, 4). Due to the significantly higher SOC contents in E860 m soils (Table 1), we can confirm the finding of earlier studies that soils with higher SOC contents are more susceptible to DOC leaching (Hope et al. 1994; Manninen et al. 2018). Our multiple regression analysis also revealed a positive correlation of the annual DOC losses with top soil SOC contents, while other variables, such as MAT, MAP, AGB and C input via manure did not further improve the prediction capacity of the regression. Since AGB and C input via manure did not influence DOC concentrations in the 140 cm soil depth, this outcome explains why annual DOC leaching losses did not differ between intensive and extensive

management. Overall studies on agricultural management impacts on DOC losses are few in number. Nevertheless, our results support similar findings in other grassland studies (Jones et al. 2006; Stergiadi et al. 2016) but differ from Bol et al. (1999) who reported that organic fertilization significantly increased DOC leaching in grassland soils at 10 cm soil depth. Significantly higher DOC concentrations in the top soil of the intensive compared to the extensive treatments (Table 2) of this study would still support this finding, but since this is not consistent with increasing soil depth, top soil effects likely cannot be generalized to whole soil profiles.

## Conclusion

In this study, effects of contrasting climate, soil and management conditions on soil DOC concentration and leaching were investigated over a 3-years period at three montane grassland sites in the TERENO Pre-Alpine observatory in S-Germany. Overall DOC concentrations and thus DOC leaching rates significantly decrease from 10, 30, 50 to 140 cm soil depth. This finding highlights, that comparing DOC leaching rates across studies differing in sampling depths can be highly biased and may explain why our leaching rates at 140 cm soil depth are lower than values reported for comparable systems with sampling depths ranging mostly between 75 and 120 cm.

In contrast to our expectation climate warming increased DOC losses only when simultaneous reductions in leachate were moderate. Still absolute increases were small ( $< 10 \text{ kg C ha}^{-1} \text{ year}^{-1}$ ) and did not change the finding that DOC leaching plays only a minor role in the C balance of grassland ecosystems as observed for current climate conditions. Plants likely play a crucial role in topsoil DOC dynamics since aboveground biomass productivity was positively correlated with the DOC concentrations in the upper 50 cm of the soil profile. However, since this correlation was not significant at 140 cm, adsorption and microbial decomposition processes in the subsoil (50–140 cm) likely provide important controls on DOC leaching rates and may explain why DOC leaching rates did not differ between different management intensities. Therefore, further studies should also include better characterization of production, consumption and transport processes along the soil

profile by e.g. stable isotope approaches and chemical and structural characterization of DOC and DON, particularly in the subsoil, which is currently under-represented in the literature.

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