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Combined effects of multifactor climate change and land-use on decomposition in temperate grassland

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ABSTRACT

Climate change is likely to alter decomposition rates through direct effects on soil biotic activity and indirect effects on litter quality with possible impacts on the global carbon budget and nutrient cycling. Currently, there is a need to study the combined effects of climatic drivers and agricultural practises on decomposition.

In an in situ litter bag experiment, we studied the effects of rainfall variability (including drought combined with heavy rain pulses as well as regular irrigation) interacting with winter warming and increased winter precipitation and with changes in cutting frequency, on decomposition in a temperate grassland. Litter bags contained mixed and species-specific litter of all different climate and land-use manipulations and were placed within the plots of litter origin. Moreover, we aimed to disentangle the causes of changes in decomposition by investigating two further approaches: Firstly, we studied the effects of changes in leaf chemicals as a result of the manipulations by removing litter from the experiment that has been pre-exposed to the manipulations before placing it on an untreated standard plot outside the experiment. Secondly, we assessed the effects of changes in soil faunal activity by investigating the decomposition of standard material under differing rainfall variability.

As a result, decomposition was reduced when litter bags were exposed to drought for six weeks within an 11 months period. Neither additional winter rain nor winter warming had an effect on decomposition, likely because winter warming reduced snow cover and increased variability of surface temperatures. Climate manipulations did not change litter quality. Furthermore, decomposition on the untreated standard plot was not affected by the climate manipulations that the litter was previously exposed to. Thus, reduced decomposition under extreme rainfall variability and drought may mainly be caused by a decrease in soil biotic activity, as indicated by reduced decomposition of standard material during drought.

More frequent cutting strongly stimulated decomposition, however, this stimulating effect was absent under extreme rainfall variability including drought. The stimulation of decomposition under more frequent cutting was attributed to changes in litter quality, namely a decrease in C/N ratio. Accordingly, litter from more frequently cut communities decomposed faster on the untreated control plot outside the experiment.

Projected increases in drought frequency and increased rainfall variability under climate change may inhibit decomposition and alter nutrient and carbon cycling along with soil quality. Especially decomposition in frequently cut grassland appears vulnerable towards drought.

1. Introduction

Litter decomposition plays a major role in the carbon budget as well as for nutrient cycling in terrestrial ecosystems (Aerts, 1997; Chapin et al., 2002). Decomposition processes are mainly governed by the three factors (i) climate, (ii) leaf litter quality and (iii) the

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composition and activity of the decomposer community (Swift et al., 1979; Lavelle et al., 1993; Aerts, 1997). Thus, climate change is likely to alter litter decomposition, where changes in litter decomposition rates might severely affect soil quality along with carbon and nutrient cycling. As grassland biomes store up to 30% of soil carbon worldwide (Risch et al., 2007), effects of climatic change on decomposition in grasslands are of major interest. Positive feedback processes may intensify warming due to rising CO_2 levels (Bontti et al., 2009). Climate change not only means a gradual warming trend, but also increases intra-annual rainfall variability, causing longer dry periods and more intense heavy rain spells (Meehl et al., 2007). Moreover, within Central Europe, warming will be most pronounced during winter, during which the overall precipitation amount is also projected to increase (Christensen et al., 2007).

Changing climate is likely to alter decomposition processes through short term changes in soil moisture or temperature which directly affect soil biological processes, including microbial and soil community composition and activity (Hobbie, 1996; Aerts, 1997). Indirectly, climate change will alter decomposition through chemical changes of litter within single plants as well as through shifts in plant species composition (Hobbie, 1996; Aerts, 2006; Fortunel et al., 2009; Baptist et al., 2010; Osanai et al., 2012).

Reduced water availability or drought often have a negative affect on litter decomposition or soil respiration (Lensing and Wise, 2007; Risch et al., 2007; van Meeteren et al., 2008; Bontti et al., 2009; Joos et al., 2010), although these effects may be only short-termed (Kemp et al., 2003; O'Neill et al., 2003) or even non-existent (Kreyling et al., 2008). Constantly high water availability has also been shown to reduce decomposition (Tiemann and Billings, 2011; Lensing and Wise, 2007), whereas dry–wet cycles might increase decomposition (Xu et al., 2004; Miller et al., 2005). Warming has often been found to increase litter decomposition (Hobbie, 1996; van Meeteren et al., 2008; Kirwan and Blum, 2011) due to an increase in microbial and enzymatic activity (Chapin et al., 2002; Aerts, 2006; Allison and Treseder, 2011), although some studies suggest that this effect does not always occur (Giardina and Ryan, 2000; Risch et al., 2007). Furthermore, increased winter temperatures are likely to result in colder soil conditions due to a reduced layer of insulating snow (Hardy et al., 2001; Kreyling, 2010), which may even decrease decomposition. Thus, no consensus about the role of global warming on decomposition has yet emerged.

The few existing studies combining multiple climatic factors often found non-additive effects of the different factors. For instance, the combination of CO_2 enrichment and warming did not react in the same way as both factors alone on microbial biomass carbon (Andresen et al., 2010) or as temperature-dependence of decomposition depended on moisture-availability (Butenschoen et al., 2011). Thus, acceleration of decomposition caused by warming may be offset under drier conditions (Gavazov, 2010; Butenschoen et al., 2011).

Therefore, there is an urgent need to further study interactions between different climatic factors according to scenarios of future change, most importantly, the simultaneously occurring factors of warming and precipitation variability (Aerts, 2006; Butenschoen et al., 2011). Moreover, the impact of agricultural practise, such as frequency of cutting on decomposition needs to be addressed, as it may strongly alter decomposition, e.g. by changes in litter quality, due to reduced shoot–root ratio or higher nitrogen concentrations in younger leaves (Walter et al., 2012).

In order to study the combined effects of increased inter-annual rainfall variability with winter climate change scenarios and agricultural practise on decomposition, we conducted a litter bag experiment in a semi-natural grassland using different climate change scenarios and varying cutting frequencies. The grassland was subjected to summer drought followed by heavy rain pulses (extreme variability), regular irrigation (low variability) and to ambient rainfall (mid variability) in combination with winter warming, additional winter rain and two different cutting frequencies. We aimed to disentangle the causes for possible changes in decomposition, being either leaf chemical alterations or modifications in soil biotic activity, by testing decomposition in an in situ experiment as well as under standardized conditions.

Our hypotheses include:

(1) extreme rainfall variability including drought will reduce decomposition rates, whereas heavy rain pulses after drought will not have a long-term effect on decomposition
(2) more frequent cutting will stimulate decomposition, independent of summer rainfall variability, caused by more beneficial leaf chemistry, e.g. younger leaves with higher nitrogen content
(3) winter warming will increase decomposition, except for winter warming leading to a reduced layer of insulating snow and thus actually decreasing soil and surface temperatures
(4) additional winter rain will not affect decomposition as winters in Central Europe are already usually wet and decomposition should not be moisture-limited during this time

2. Material and methods

2.1. Study site and experimental setup

The study was conducted within the EVENT II experiment, which investigates the impact of inter-annual rainfall variability in combination with winter climate change and agricultural practise in temperate grasslands. The experiment was established in 2008 in a semi-natural grassland in the Ecological Botanical Garden of the University of Bayreuth, Germany, Central Europe (49°55′19″N, 11°34′55″E, 365 m asl) (Walter et al., 2012) and this study was conducted in 2010–2011, at the end of the third year of rainfall manipulations. Communities are dominated by tall grasses, especially *Alopecurus pratensis* L. (meadow foxtail) and *Arrhenatherum elatius* L. (tall oat grass). The regional climate is temperate and moderately continental.

The experimental design for this study consisted of five replications of three rainfall variability regimes applied in the vegetation periods in blocks 6 m × 4 m in size. For the manipulations of rainfall variability, the temporal distribution and the magnitude of rainfall per rainfall event in the growing season was altered, but annual rainfall amount has been constant since 2009 by applying compensation irrigations. A change of rainfall patterns, most notably an increase in the severity of drought and in the frequency of heavy rain spells, is projected under climate change, also for Central Europe and Germany (Meehl et al., 2007; Jacob, 2009). The three rainfall variability regimes were: (1) low variability, with weekly irrigation corresponding to the 30 year average amount of the respective week, ensuring a continuous water supply (low), (2) mid variability, receiving ambient rainfall plus compensation irrigations (4 times per year) to keep the annual rainfall amount constant at quarterly intervals (mid) and (3) extreme variability, subjected to a summer drought treatment, followed by heavy rain pulses (extreme). For the low variability treatment, amounts from 1971 to 2000 served as a reference (data: Foken, 2003). Missing amounts on natural precipitation were added if the weekly precipitation was less than the long-term average for the same week to ensure continuous water availability. If weekly precipitation exceeded the long-term sum, it was not deducted from the next irrigation. From September 1st 2010 until August 31st 2011 all variability treatments received 925 mm, exceeding the 30-year-average sum for this time period by 202 mm, and thus simulating a rather wet year. Table 1 lists the irrigated amounts of all compensation irrigations for the mid and extreme variability treatments.

For the extreme variability treatment, tunnel-shaped rain-out-shelters excluded natural precipitation from June 22nd until August 3rd in 2010 and from July 5th until August 16th in 2011, resulting in an extreme summer drought of 42 days, followed by two days of extreme irrigation as compensation irrigations. The PE-foil of the rain-out-shelters allowed nearly 90% of photosynthetic active radiation. Shelters were built starting at a height of 0.8 m in order to reduce microclimatic artifacts. Irrigation was applied using portable irrigation systems with a drop size and rainfall intensity comparable to natural rainfall events. Lateral surface flow was reduced by using plastic sheet pilings around all plots reaching down to a depth of 0.2–0.25 m.

In each rainfall variability block four subplots of 1.5 m × 1.5 m were nested, in which differing winter climate change scenarios and cutting frequencies were executed ($n = 60$).

These intra-block manipulations mimicked common agricultural practise (two cuts: 2 × R (July and September) versus four cuts: 4 × R (May, July, August, September)). Furthermore they depicted projected winter climate change for Germany, most notably an increase in winter precipitation and temperatures (Jacob, 2009; Zebisch et al., 2012). Aboveground temperatures from October until April were increased by on average 1.1 °C at 0.05 m height and by 1.3 °C in the soil using IR-heating lamps at a height of 1 m in the warmed plots (2 × W). This change falls into the projected range of warming of the IPCC B1-scenario depicting rather moderate warming, compared to more emission intense scenarios (Meehl et al., 2007). The additional winter rain was applied in four monthly steps from November to February. One nested subplot was cut twice per year with no further manipulation (2×). Table 2 summarizes all rainfall variability regimes and the nested subplot scenarios.

2.2. Soil moisture and temperature

Soil moisture was logged every hour using FD-sensors in each treatment combination (ECH_2O, Decagon devices, Pullman, USA) ($n = 5$/treatment combination). Each sensor measured the soil moisture between −2 and −7 cm. According to root length data, the majority of root biomass can be found within the uppermost 5 cm of the soil. Fig. 1 shows the course of soil moisture over the experimental period with daily averaged values. The temperature for each rainfall variability treatment in warmed and un-warmed plots was measured at 10-min intervals by thermistors (B57863-S302-F40, EPCOS) and logged as an hourly average by a data-logger (dl2, Delta) at 2 cm depth (soil) and at 5 cm height. Fig. 2 shows the course of temperature and snow depth during winter for plots warmed and not warmed from October until April.

Table 1
Compensation irrigation applied on mid- and extreme variability treatments during the experimental period to ensure an overall identical precipitation sum in all three rainfall variability regimes. Compensation irrigation amounts were adjusted to the amount of the low variability treatment, which was watered weekly and thus did not receive compensating irrigations.

Date	Rainfall variability regime	
	Mid	Extreme
September 27th 2010	17.5	17.5
May 23rd 2011	52.9	52.9
July 4th 2011	26.3	26.3
August 15th 2011	33.9	229.9[a]

[a] Applied on two consecutive days.

2.3. Sampling design for the litter bags and chemical analyses

In order to investigate the effects of rainfall variability regime in combination with the different winter climate change and cutting scenarios, biomass sampling for the litter bags was conducted on September 6th and 7th in 2010. We obtained mixed samples by cutting four different circular areas, 0.20 m in diameter in each plot ($n = 60$). To estimate effects of changes in leaf chemicals caused by different cutting regimes and to disentangle intra- from inter-specific alterations, we sampled a single grass species, *A. pratensis* of the subplots 2 × R and 4 × R. All samples were oven-dried for 72 h at 40 °C. After drying, 3 g ± 0.03 g were weighed into nylon mesh bags (10 cm × 20 cm) with a mesh size of 1 mm and the exact weight was recorded. This allows fungi, bacteria, microfauna and most of the mesofauna to attack the litter (Chapin et al., 2002). Mixed samples and *A. pratensis* out of 2 × R and 4 × R plots were ground in a ball mill and analysed for carbon (C) and nitrogen (N) with an elemental analyser (Thermo Quest Flash EA 1112), as C/N ratio often correlates well with decomposition rates, especially in grassland (Chapin et al., 2002; Lambers et al., 2008).

2.4. Placement of the litter bags

Two mixed litter bags per plot were placed in the plots the litter was sampled from in late September. Bags were placed on the vegetation that was cut to the ground and attached to the ground using two plastic coated wires placed diagonally over the litter bag. Litter bags were removed in late August 2011 (after 11 month) and thus received a direct summer drought followed by a rewetting pulse in 2011.

In order to separate the effects of rainfall variability regime and cutting frequency on decomposition, mixed samples out of all rainfall variability regimes and the subplots 2 × R and 4 × R ($n = 30$) were placed on a standardized, untreated, mulched plot outside of the experimental site. Distinguishing chemical effects caused by intra-specific (variations of leaf chemicals within single plant species) or inter-specific (variations in leaf chemicals due to changes in plant community composition) alteration the *A. pratensis* samples were also placed on this untreated plot ($n = 30$). Those bags were removed in May 2011, after 8 months of decomposition.

After retrieval, all bags were dried for 72 h at 40 °C and stored in air tight containers with silica gel until they were weighed on a micro-balance. Percentage of dry weight loss was calculated as a proxy for decomposition. Table 2 gives an overview of the sampling design and placement of the litter bags.

2.5. Soil biotic activity

A bait-lamina test (Kratz, 1998) was performed to measure the effects of rainfall variability on soil biotic activity in August 2011, during the drought period in the extreme variability treatment. This approach complements the decomposition trial by investigating the treatment effects using a standard material (Thörne, 1990). Each bait-lamina stick (*Terra Protecta*® GmbH, Berlin, Germany) contained 16 baits which consisted of a wet mixture of cellulose, bran flakes and activated coal (70:27:3). Within each rainfall variability regime, only the 2×, 2 × R and 2 × W subplots were included, as in the preceding year, cutting frequency was shown not to affect soil enzymatic activity (see supplemental information). In each plot, two baited sticks were placed vertically into soil and remained there for 14 days. At the end of the exposure period each stick was carefully removed from soil, placed into plastic bags and stored in a freezer at −30 °C until further analyses were performed. Perforated baits of the cleaned bait sticks were recorded and expressed as percentage of eaten baits per plot.

Table 2
Descriptions and abbreviations of applied rainfall variability regimes during the vegetation period and of the nested winter climate change and cutting frequency scenarios and sampling design for the litter bags.

Variability	Description	Mowing frequency/ winter climate	Description	Mixed litter bags within experiment[a]	Untreated standard plot[b]
Low	Weekly irrigation with 30-year average	2×	Mown twice/year, no further manipulation	2 Mixed, Sept–August	
		2 × R	Mown twice, 60 mm winter rain added	2 Mixed, Sept–August	2 Mixed, 2 *A. pratensis*
		4 × R	Mown four times, 60 mm winter rain added	2 Mixed, Sept–August	2 Mixed, 2 *A. pratensis*
		2 × W	Mown twice, winter warming October–April	2 Mixed, Sept–August	
Mid	Ambient rainfall with compensation irrigation	2×	Mown twice/year, no further manipulation	2 Mixed, Sept–August	
		2 × R	Mown twice, 60 mm winter rain added	2 Mixed, Sept–August	2 Mixed, 2 *A. pratensis*
		4 × R	Mown four times, 60 mm winter rain added	2 Mixed, Sept–August	2 Mixed, 2 *A. pratensis*
		2 × W	Mown twice, winter warming October–April	2 Mixed, Sept–August	
Extreme	42 Days summer drought followed by extreme compensation irrigation pulses	2×	Mown twice/year, no further manipulation	2 Mixed, Sept–August	
		2 × R	Mown twice, 60 mm winter rain added	2 Mixed, Sept–August	2 Mixed, 2 *A. pratensis*
		4 × R	Mown four times, 60 mm winter rain added	2 Mixed, Sept–August	2 Mixed, 2 *A. pratensis*
		2 × W	Mown twice, winter warming October–April	2 Mixed, Sept–August	

[a] Bags were sampled from and placed on the respective treatment combination.

[b] Bags were sampled from the respective treatment combination and placed on untreated plot under standard conditions to disentangle causes for changes in decomposition rates (litter quality or soil biotic activity).

2.6. Statistical analysis

In order to test for significant effects of summer rainfall variability in the differing winter climate change and cutting frequency scenarios, two-factorial ANOVA with the fixed factors “rainfall variability regime” and “winter climate change and cutting scenario” were performed. As each rainfall variability block occurred just once in each row and each column of the design, we included the row and the column of the weather treatment blocks as random factors in our linear mixed effect model. This also implements the nesting of winter climate change/cutting scenario within the rainfall variability blocks in the model, as one block with its corresponding and unique row and column combination includes four values for the response variable (Faraway, 2006). Tukey HSD tests were calculated for post-hoc analysis in order to determine differences between rainfall variability treatments. As the subplot scenarios were not all directly comparable with each other we only included the directly comparable data in further mixed models for post-hoc analysis if the effect of subplot scenarios or the interaction of subplot scenarios with rainfall variability was significant. This avoids unnecessary comparisons. Scenarios that were directly comparable as only one factor is varied were: (i) 2× + wr with 4× + wr to test for effects of cutting frequency, (ii) 2× + wr with 2× to test for effects of winter rain and (iii) 2× + ww with 2× to test for effects of winter warming. These data were analysed for the effects of winter climate change and cutting frequency and of combined effects of those with rainfall variability.

All statistical analyses were performed using R 2.11.0 (R Development Core Team, 2010). For mixed effect models we used the software package lme4 (Bates, 2010). In addition, the package nlme (Pinheiro et al., 2008) was used to run the Tukey HSD tests. Significance levels in mixed effect models were evaluated by Markov Chain Monte Carlo sampling of 1000 permutations, using the software package language R (Baayen, 2009), as lme4 does not give significance values.

3. Results

During the drought manipulation in the extreme variability treatment in 2011, soil moisture was by 28% and by 34% lower than in the mid- and low variability treatments, respectively (Fig. 1). The compensating heavy rain spells after the drought treatment elevated soil moisture levels rapidly, but did not result in higher soil moisture than in the other two rainfall variability manipulations. Over the entire experimental period, the low variability treatment had the highest average soil moisture, whereby soil moisture in the extreme and mid variability treatments was 12% and 7% lower, respectively. The average soil moisture of the subplot scenarios did not differ by more than 5% during the whole experimental period.

The winter warming treatment increased temperature slightly by 1.1 °C on average, but also decreased temperature minima and resulted in a 16% increase of frost days at 5 cm height and in an increase of temperature variability (Fig. 2).

3.1. Effects of summer rainfall variability regime on decomposition and soil biotic activity

Litter decomposition over the 11 months period was strongly affected by the extreme variability treatment, as those samples decomposed significantly slower when compared to mid and low rainfall variability (overall effect of rainfall variability: $F(2,83) = 5.5$; $p = 0.006$; Fig. 3A). Furthermore, extreme rainfall variability significantly reduced soil biotic activity during the drought period as measured by the bait-lamina test when compared to low and mid rainfall variability (overall rainfall variability effect $F(2,41) = 11.0$, $p > 0.001$; Fig. 3B).

3.2. Effects of cutting frequency and winter climate change on decomposition

Generally, cutting frequency strongly affected the rate of decomposition ($F(1,41) = 34.1$; $p < 0.001$), with litter cut four times per year decomposing significantly faster than litter cut only twice (Fig. 4). The stimulating effect of more frequent cutting was dependent on the rainfall variability regime (significant interaction $F(2,41) = 7.9$; $p = 0.001$): This effect was not significant in the extreme variability treatment, but highly significant for the mid and low rainfall variability treatments ($p < 0.001$) (Fig. 4). Thus, litter in plots cut four times per year decomposed faster under mid and low rainfall variability ($p < 0.001$ and $p = 0.002$, respectively) when compared to extreme variability. Neither winter warming nor additional winter rain affected decomposition (Fig. 5A, B).

3.3. Decomposition under common standard conditions and leaf chemical traits

Pre-exposure of *A. pratensis* and the mixed litter to rainfall variability did not affect their decomposition in the untreated standard plot ($F(2,40) = 0.9$; $p = 0.43$ and $F(2,45) = 2.9$; $p = 0.06$, respectively (data not shown)). Cutting frequency had a strong

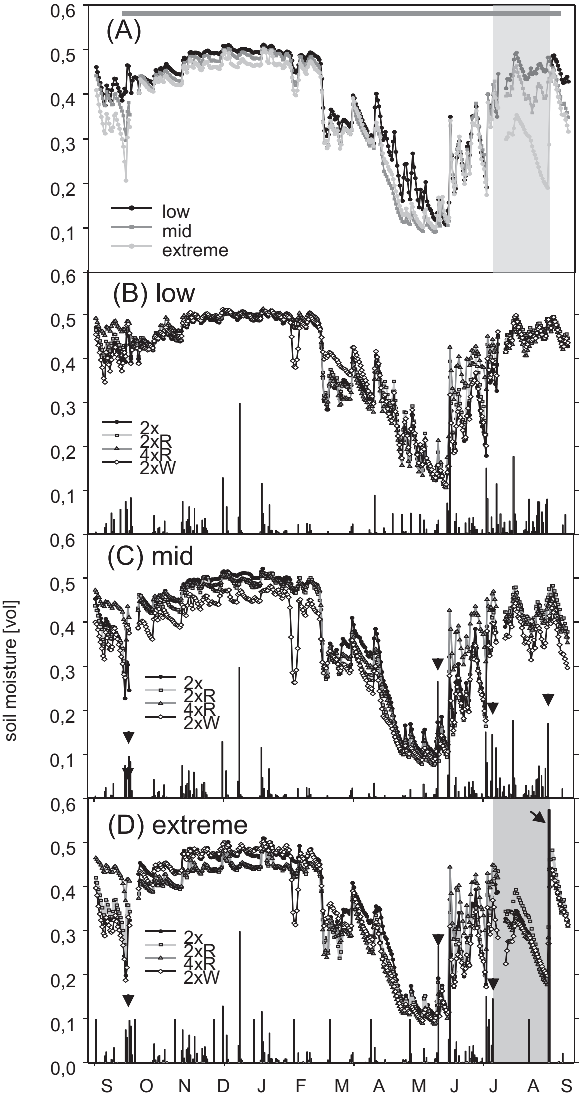

Fig. 1. Soil moisture over the experimental period in the low, mid and extreme variability treatments averaged over all subtreatments (A) and in the winter climate change and cutting frequency manipulations within the low (B), mid (C), and extreme (D) rainfall variability treatments (black circle: cut twice (2×); white square: cut twice with additional winter rain (2 × R); dark grey triangle: cut four times with winter rain (4 × R); white diamond: cut twice and warmed during winter (2 × W)). The grey area in (D) marks the duration of the extreme drought. Black arrows in (C) and (D) mark the compensation irrigations. The grey vertical line in (A) shows the exposure time of the litter bags.

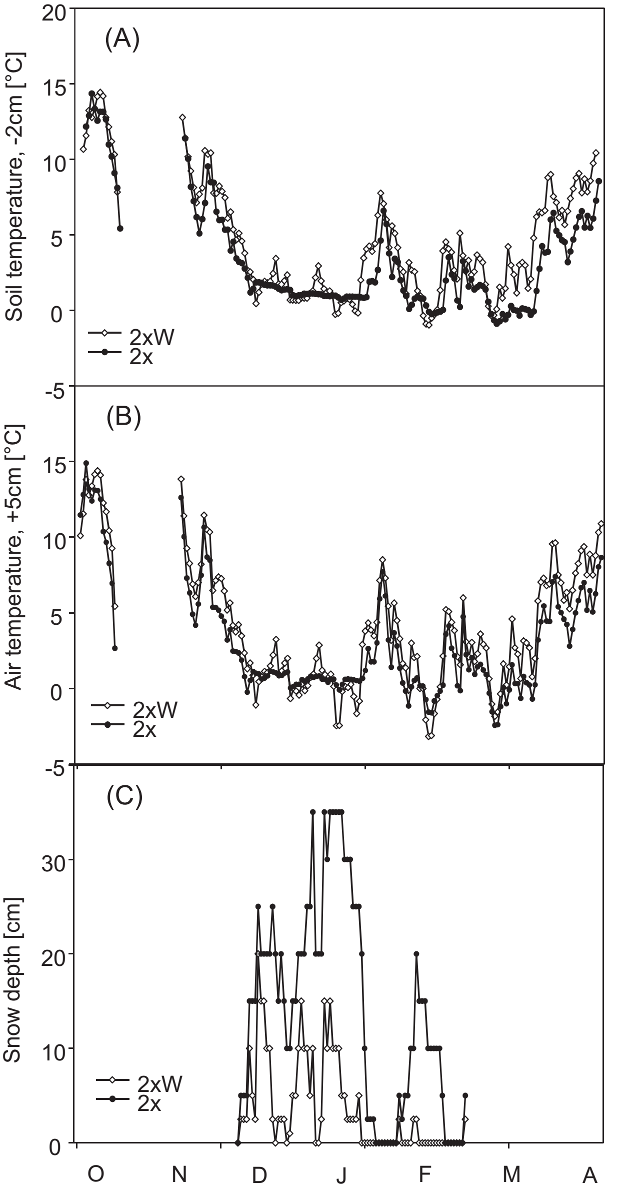

Fig. 2. Soil temperature at 2 cm depth (A), air temperature at 5 cm height (B) and snow depth (C) in the plots warmed (open circles) and not warmed (black circles) during winter. Averages over the low, mid and extreme rainfall variability treatments are given. Temperature data between October 16 and November 3 are missing due to technical failure.

effect on decomposition in the untreated control plot, as *A. pratensis* leaves and mixed samples from plots cut four times per year decomposed significantly faster than those from plots cut only twice per year ($F(1,40) = 27.10$; $p < 0.001$ and $F(1,45) = 35.9$; $p < 0.001$, respectively; Fig. 6A, B).

C/N ratio was the best predictor for decomposition of mixed litter (Pearson's coefficient = −0.51, $p = 0.007$) and *A. pratensis* litter (Pearson's coefficient: −0.57, $p = 0.003$). Rainfall variability pre-exposure did not alter C/N ratio of the target grass and of the mixed samples several weeks after the drought in the extreme variability treatment had been applied ($F(2,24) = 1.6$; $p = 0.22$ and $F(2,24) = 1.8$; $p = 0.19$; data not shown). More frequent cutting decreased the C/N ratio of the grass by 24% ($F(1,22) = 13.1$; $p = 0.002$) and C/N ratio of the mixed samples by 25% ($F(1,22) = 27.0$; $p < 0.001$; Fig. 6C, D).

4. Discussion

The manipulation of rainfall variability led to moderately higher soil moisture in the low variability treatment compared to the other treatments and to a strong reduction of soil moisture during the drought in the extreme variability treatment, which was quickly restored after the extreme rewetting pulses. The extreme compensation irrigation, however, did not increase the soil moisture level such that it reached the values for the mid- and low rainfall variability manipulations. Extreme rainfall pulses after dry periods may not be as efficient in increasing soil moisture due to increased runoff and a reduced water holding capacity of soils (Carminati et al., 2010; Walter et al., 2012). Increased winter precipitation did not result in increased soil moisture levels, possibly because soil moisture levels are already very high and saturated during winter.

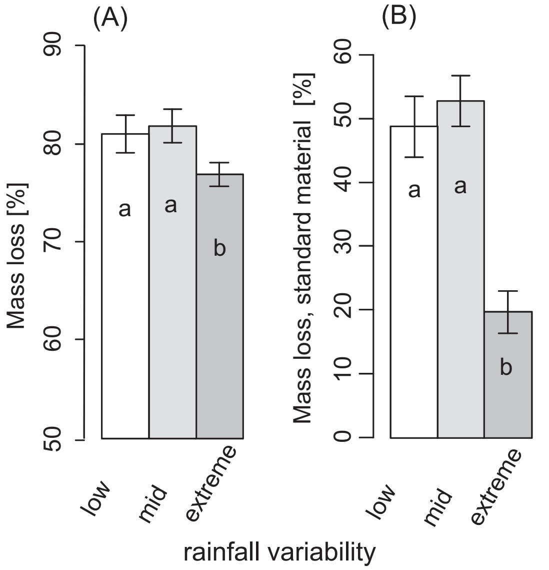

Fig. 3. Effects of rainfall variability on mass loss of mixed litter obtained from and placed within the experiment (A) (white bars: low rainfall variability (weekly irrigation), light grey bars: mid rainfall variability; dark grey bars: extreme rainfall variability (summer drought plus heavy rain)). The bait-lamina sticks including standard material (B) were only placed within the experiment for two weeks during the drought period in the extreme variability treatment in 2011. Different letters indicate significant differences ($p < 0.05$).

Winter warming resulted in a moderate increase in the average temperature and temperature variability and a decrease in snow depth.

4.1. Effects of summer rainfall variability on decomposition

The exposure of litter bags to extreme rainfall variability, including drought for only six weeks within an 11 month period, inhibited decomposition. This reduction in decomposition under extreme rainfall variability was not caused by changes in leaf litter C or N, but probably rather by a decrease in soil microbial and faunal activity under drought, as indicated by the bait-lamina test. Similar results have been found in other studies (Liao et al., 2002; Kemp et al., 2003; Emmett et al., 2004; Lensing and Wise, 2007; Risch et al., 2007; van Meeteren et al., 2008; Bontti et al., 2009; Reed et al., 2009; Andresen et al., 2010; Joos et al., 2010). Contrary to other studies (Sorensen, 1974; Xu et al., 2004; Miller et al., 2005),

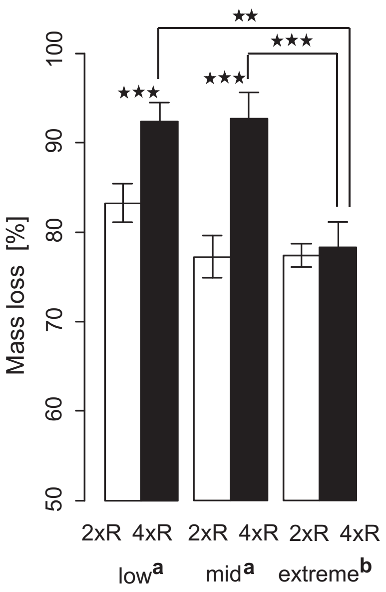

Fig. 4. Interacting effects of cutting frequency and rainfall variability on litter mass loss of mixed litter (black bars: cut four times per year, white bars: cut twice). Asterisks display significant differences between neighbouring bars or the bars connected with braces (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$). Different superscript letters above the rainfall variability manipulations indicate significant differences between the manipulations.

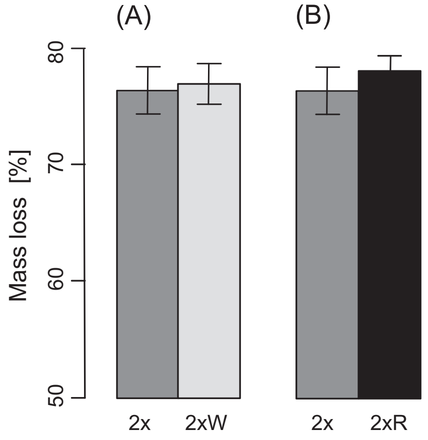

Fig. 5. Effects of winter warming (A) and of winter rain (B) on litter mass loss. Dark grey bars: no winter rain or winter warming; light grey bar: warmed from October until April; black bar: 60 mm additional winter rain.

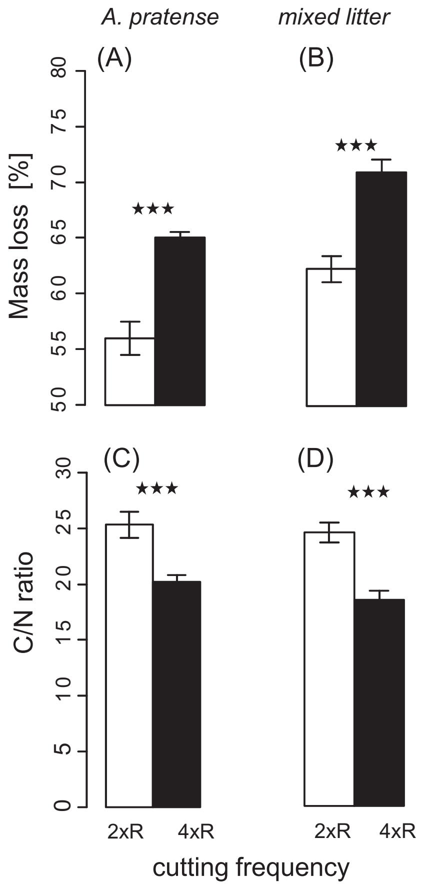

Fig. 6. Effects of cutting frequency on litter mass loss of *A. pratensis* (A) and of mixed litter (B) under ambient standard conditions, and effects on C/N ratio of *A. pratensis* (C) and of mixed litter (D). Asterisks indicate significant differences between the bars (***: $p < 0.001$).

the rewetting pulses after drought did not stimulate decomposition, at least not sufficiently to compensate for the decrease in decomposition during drought. It is possible that the pulses after drought were not sufficient, due to increased runoff or evapotranspiration. This is indicated by the failure of rewetting to increase soil moisture above the levels of the other two treatments, which did not receive the heavy rain pulses.

As decomposition should accelerate under warm and moist (but not water-logged) conditions (Chapin et al., 2002; Aerts, 2006) we expected decomposition to be fastest in communities that were irrigated weekly during summer (the low variability treatment). However, contrary to our expectations, weekly irrigations did not promote decomposition. Accordingly, Lensing and Wise (2007) report higher decomposition under ambient rainfall conditions than under irrigation. Tiemann and Billings (2011) show decreases in soil respiration when subject to regular irrigation when compared to soil respiration under higher soil moisture variability. Guenet et al. (2012) find the highest soil enzymatic activity under intermediate moisture levels, which might relate to our mid rainfall variability. Furthermore, the microbial and fungal community became less abundant and diverse under regular watering (Cornejo et al., 1994; Hawkes et al., 2011), which might also inhibit a promotion of decomposition under regularly watered conditions.

4.2. *Effects of cutting frequency and interactions with summer rainfall variability*

As expected, more frequent cutting promoted decomposition. This can be explained by a lower C/N ratio and thus a faster decomposition in community mixtures as well as in the single grass species, which has been shown for decomposition under untreated standard conditions. Increased N concentrations in more frequently cut leaves have been found in other studies as well and might be attributed to a lower shoot–root ratio, younger leaves and higher N-mineralization (Turner et al., 1993; Green and Detling, 2000; Maron and Jeffries, 2001; Walter et al., 2012). Leaf chemical changes in the single grass species showed the same trend and were of the same magnitude as changes in mixed litter. Thus, contrasting other studies (Hobbie, 1996; Aerts, 2006; Wardle et al., 2009), the chemical changes of mixed litter were more likely caused by intraspecific changes and not by changes in plant community composition. Our data related to plant community composition in the first three years of the experiment (Bray–Curtis dissimilarity) did not show shifts in community composition caused by the rainfall variability manipulations included in this study (unpublished data). Shifts in functional group composition or diversity often take place over longer time scales (Bardgett and Wardle, 2010).

Cutting frequency also strongly interacted with summer rainfall variability as decomposition was not stimulated by cutting four times per year when litter was derived out of the extreme rainfall variability regime. Unlike the general accelerating effect of more frequent cutting on decomposition, the reduction of this accelerating effect under extreme rainfall variability cannot be explained by changes in leaf chemicals. It is not mirrored in C/N ratio alterations and did not occur on the untreated standard plot. In the preceding year 2009 it was shown that cutting frequency does not alter soil enzymatic activity (see SI for an example), but we do not have data from our experimental period. Microclimatic conditions might react differently under drought in more frequently cut communities. During summer, soil moisture was often slightly higher in more frequently cut communities, but not during the drought treatment. This might have rendered the microbial community more vulnerable towards drought. Further studies should investigate long-term microbial activity and microclimate during and after drought in more and less frequently cut meadows. Our findings imply that decomposition in more frequently cut grassland might be more responsive to drought conditions than less frequently cut grasslands. Such an impairment of nutrient turnover may lead to reductions in soil quality and thus also to reductions in productivity and forage quality under more frequent drought events, especially in more intensively managed grassland.

4.3. *Effects of winter warming and winter rain on decomposition*

A lack of a stimulating affect of warming on decomposition is often due to a concomitant decrease in soil moisture (Aerts, 2006; Bontti et al., 2009; Gavazov, 2010). Our soil moisture data also show a sudden drop under winter warming in February (Fig. 1). However, this drop was not caused by increased evapotranspiration, but probably by a prolonged soil frost in the warmed communities, due to melting of the snow cover in the warmed plots, when compared to un-warmed plots (Fig. 2). FD sensors only detect plant available liquid water. Our warming treatment increased temperature slightly by 1.1 °C on average, but also decreased temperature minima and resulted in a 16% increase of frost days at 5 cm height, again likely due to a lack of insulation caused by snow-melt. These findings support evidence that winter warming might well lead to an *increase* in frost stress for many plants, accompanied by changes in soil cation balance and acidity (Groffman et al., 2001; Gu et al., 2008; Kreyling, 2010; Comerford et al., 2012) and might lead to an increase in soil or surface temperature variability during winter. This might explain the lack of an affect on decomposition in our winter warming manipulation. However, it should be noted that the moderate warming increment of 1.1 °C on average, may not have been sufficient to stimulate a decomposition effect.

Concerns that global warming might lead to a stimulation of decomposition and soil respiration and thus to increases of carbon loss and positive feedback processes on climate, especially under cold conditions (Kirschbaum, 1995; Aerts, 2006) seem not to be generally justified regarding temperate grasslands during winter (Giardina and Ryan, 2000). Our results, however, imply that snow cover might be critical for this conclusion. The lack of a stimulating effect of additional winter rain on decomposition shows that moisture can only stimulate decay processes when it is a limiting factor, which is not the case during Central European winters. With regard to decomposition, the very likely trend towards wetter winters in temperate regions (Christensen et al., 2007) consequently appears unimportant.

5. Conclusions

We show that even a very short drought followed by heavy rain pulses relative to the exposure period decreases the litter mass loss rate by 5%. Especially decomposition in more frequently cut grassland was vulnerable to drought. Drier climatic conditions under global warming could, thus, slow down nutrient cycling and alter soil–carbon balance in more intensively managed grasslands. Changes in climatic variables directly affected decomposition through changes of soil biotic activity and not through litter quality alterations, as neither C/N ratio nor decomposition under untreated standard conditions were altered through litter pre-exposure to rainfall variability. Contrastingly, the stimulation of decomposition of more frequent cutting was largely explained by changes in litter quality, most notably a decrease in the C/N ratio. Surprisingly, changes in winter climate and especially winter warming had no stimulating effect on decomposition. Either the achieved warming was too moderate to stimulate decomposition, or the reduced snow cover and the concomitant increases in temperature variability and soil frost offset a stimulating effect of warming on litter decomposition. We conclude that the interplay between winter warming and decomposition is dependent on snow cover for temperate regions. To conclude, although grassland decomposition and soil biotic activity seemed to be quite resistant towards changes in climatic variables, certain future projections, such as increased drought frequency or continued winter warming beyond the cooling capacity of missing snow cover could necessitate an adaptation of agricultural routines to sustain soil quality and productivity.

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.soilbio.2013.01.018.

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