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Effects of elevated temperature, elevated CO_2 and fertilization on quality and subsequent decomposition of silver birch leaf litter

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A B S T R A C T

We examined the quality and decomposition of naturally abscised leaves of silver birch (*Betula pendula*) seedlings subjected to three different levels of fertilization under ambient and elevated levels of temperature and CO_2. At the end of the second growing season, the chemical composition of the litter collected from the seedlings was analyzed. Whole-leaf samples from pooled litter from each of the four replicates from each treatment were put in mesh bags and transferred to ambient climate in the field. The remaining mass of litter was measured by sampling bags in May and October throughout the four-year incubation period. Fertilization with all nutrients decreased the initial carbon and tannin contents of litter, and increased the proportion of the fast-decomposing fraction, but still fertilization slowed down the decomposition of this fraction. Initially, the estimated proportion of the fast-decomposing fraction was smallest in elevated CO_2 + temperature, and largest in ambient climate. During decomposition, elevated growth-temperature slowed down decomposition of the fast fraction under ambient CO_2 but increased it under elevated CO_2. The changes in litter decomposition rates found over four years were not very large. However, we conclude that the interactions of different factors lead to different results than if the factors had been studied separately, and future studies should take interactions into account.

1. Introduction

Wide-ranging impacts of increasing atmospheric carbon dioxide (CO_2) concentration and globally rising temperature may have an effect on the decay process and, consequently, carbon cycle in terrestrial ecosystems (Fierer et al., 2005; IPCC, 2007; Sokolov et al., 2008).

The physiology of leaves of plants during their life cycle (from bud development to abscission) is directly and indirectly affected by climate. It is well known that elevation in atmospheric temperature or CO_2 concentration separately stimulates the primary and secondary metabolism in the green leaves of trees, including silver birch (*Betula pendula* Roth) (e.g. Lavola and Julkunen-Tiitto, 1994; Rey and Jarvis, 1997; Lavola et al., 2000; Kellomäki and Wang, 2001; Kuokkanen et al., 2003; Kasurinen et al., 2006). In many plant species, the structural and non-structural carbon (such as cellulose, hemicellulose, lignin, phenolic compounds and starch) in leaves is reported to increase with increasing CO_2 (e.g. Strain and Bazzaz, 1983; Cotrufo et al., 1994; Lavola and Julkunen-Tiitto, 1994; Cotrufo and Ineson, 1996; Lavola et al., 2000; Blaschke et al., 2002; Heagle et al., 2002; Körner, 2003; Kasurinen et al., 2006, 2007). Rising temperature has also led to increased contents of lignin and cellulose (Ford et al., 1979), and accumulation of sucrose (Lafta and Lorenzen, 1995) in foliage.

Climate change may affect nutrient availability by regulating soil processes. A warmer climate may accelerate weathering and erosion, as well as mineralization rates of organic matter in the soil (Hättenschwiler et al., 1996; Mäkipää et al., 1999; Zhang and Nearing, 2005). At the same time human activities may increase atmospheric deposition of nitrogen (Galloway et al., 2004). This combination of processes may enhance nutrient availability from soil, and thereby increase the biomass growth in plants (Mäkipää et al., 1999). However, increased nutrient availability tends to lower the production of carbon based secondary compounds in leaves of trees (Lavola and Julkunen-Tiitto, 1994; Keinänen et al., 1999; Laitinen et al., 2005) inducing an inverse response compared to that

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of increasing atmospheric CO_2. In either case, the tissue C:N ratio is altered; it is either increased by elevated CO_2, or decreased by increased N availability (Cotrufo et al., 1994; Aerts et al., 1995; Pregitzer et al., 1995; Salt et al., 1995; Cotrufo and Ineson, 1996; Kasurinen et al., 2006). The roles of nutrients other than N are much less understood.

Earlier studies suggest that the direct effect of CO_2 on the chemical composition of senescent leaves can range from inconsequential to definite (King et al., 2001; Tuchman et al., 2002). Since soluble non-structural carbohydrates are transported from leaves to the other plant parts in late summer (Piispanen and Saranpää, 2001; Wong et al., 2003), these conflicting data may be a consequence of the response of structural carbon to increased atmospheric CO_2: for example, the content of lignin in leaves of a range of plants subjected to CO_2 elevation has been found to decrease or increase (Cotrufo and Ineson, 1996; Poorter et al., 1997). Moreover, as stated by Zvereva and Kozlov (2006), there is little information about the combined effects of elevated temperature and CO_2 on the characteristics of leaves in different phases of their development, and the available knowledge is restricted to only a few plant species. Much less is known about the quality and subsequent decomposition of leaf litter that is developed under the combination of elevated temperature and CO_2.

The initial composition of abscised leaves affects the activity of microorganisms and their abundance in the various soil types, either accelerating or slowing down the decomposition (Hättenschwiler and Bretscher, 2001). There is evidence that the consumption of litter by decomposers decreases, when the concentrations of polyphenols and tannins are high in the decaying leaf material (Harrison, 1971). The elemental composition in litter may also affect the decomposition. The contents of nitrogen and carbon in decaying matter affect the consumption by the microorganisms (Rosswall, 1982; Teuben, 1991; Hodge et al., 2000). The initial rate of decay has been reported to be fast especially in litter with high nitrogen content and low C:N ratio, but slow in litter with high carbon content and high C:N ratio (Hunt et al., 1988; Cotrufo and Ineson, 1996; Aerts and de Caluwe, 1997).

One of the greatest changes that increasing atmospheric CO_2 and nitrogen availability may cause is the increase in net primary production, which will lead to increasing amounts of litter (Liu et al., 2009). In many regions, increasing temperatures also lead to increasing primary production. However, if there are major changes in litter quality due to global change, the rate of decomposition can also be affected (Parsons et al., 2008). If litter decomposition rates are strongly reduced because of changes in litter quality, organic matter may accumulate, nutrient release may slow down, and the increased production may not be sustainable (Knops et al., 2007). Our knowledge about the effects of CO_2, temperature, and nutrient availability is largely based on single-factor experiments. Yet all these changes – together with others, such as changes in water availability and atmospheric O_3 – will occur simultaneously, and the outcome is determined by multiple factors together. In this study, we aim to explore the interactions of three major factors of environmental change.

When studying decomposing litter, fractions are sometimes defined such as labile, intermediate and recalcitrant litter (e.g. Coûteaux et al., 1998). These fractions represent different decay rates: fast, intermediate, and very slow. Depending on the assumed number of litter fractions, different models have been fitted to data: single, double and triple negative exponentials (Adair et al., 2008; Harmon et al., 2009). In such models the relative initial size of each litter fraction and the decay rate constants for each fraction are estimated.

Within the above context, we examined the quality and the decomposition of leaf litter produced in silver birch seedlings under varying combinations of temperature, CO_2, and fertilization treatments. Our aims were to determine

(i) if the quality of the leaf litter obtained from silver birch seedlings is changed by the effects of elevated temperature or elevated CO_2, high availability of nutrients, or by their interactions, and
(ii) if the decomposition rate is altered by the chemical properties of silver birch leaf litter, i.e. by altered tannin content, by altered contents of carbon and nitrogen, or by altered C:N ratio.
(iii) if these changes can be satisfactorily described by a double negative exponential model.

2. Materials and methods

2.1. Plant material and treatments

The plant material originated from seeds collected in a naturally regenerated silver birch (*B. pendula*) stand in eastern Finland (63° 36′ N, 29° 43′ E). In early June 1999, the seeds were sown in seedling trays in an unheated greenhouse. In mid-June 1999, the seedlings (altogether 768 plants) were transplanted into Enso trays (in each tray altogether 16 cells, individual cell volume 0.3 dm^3) filled with unfertilized commercial peat (VAPO, Vapo Oy, Jyväskylä, Finland). After planting, the seedlings were transferred to climate controlled closed-top chambers at the Mekrijärvi Research Station (62° 47′ N, 30° 58′ E), University of Joensuu, Finland. The chambers (in total 16 with an internal volume of 19.3 m^3 and ground area of 5.2 m^2 in each) were built in the field, each of them enclosing a Scots pine sapling (*Pinus sylvestris* L.) with a mean height of 4 m.

The climatic treatments in the chambers included combinations of ambient or elevated temperature with ambient or elevated CO_2. The ambient CO_2 concentration was set at 360 μmol mol^{-1}, and the elevated average was set at 720 μmol mol^{-1}. The ambient temperature followed the changes in the natural temperature outside the chambers. During the growth period of the seedlings, from June to September, the elevated temperature was set at 2 °C above the ambient temperature. The specific elevations of temperature and CO_2 were automatically adjusted inside the chambers by a computer-controlled heating and cooling system together with a set of magneto-electric valves controlling the supply of pure CO_2. These treatments were designed to correspond to the climate scenario (Kellomäki and Väisänen, 1997) predicting double atmospheric CO_2 concentration from the present to the year 2080. Each of the treatments was replicated in four different chambers. A more detailed description of the chamber system is given in Kellomäki et al. (2000).

The seedling trays were positioned on the metal mesh footboards bolted onto the chamber walls at 50 cm above ground level. The location of the trays was changed weekly in order to reduce the effect of within-chamber variation. The seedlings grew in the chambers in trays throughout the experiment from mid-June 1999 to the end of the growing season of 2000. The experiment with the seedlings was implemented following a split-plot design with completely randomized main plots. The main plot treatment was climate (consisting of temperature and CO_2), under which the subplot treatment was fertilization. The fertilization treatment had three different levels (low, moderate and high), each applied to 16 seedlings per chamber. The fertilizers used contained all essential nutrients. Under the low fertilizer level (0 kg N ha^{-1} $year^{-1}$), the peat was the only source of nutrients for the seedlings. The moderate fertilizer level (150 kg N ha^{-1} $year^{-1}$, 96 mg N per seedling) was set to correspond to the average amount of nutrients used in Finnish forest nurseries for birch seedlings (Juntunen and Rikala, 2001). The high fertilizer level (500 kg N ha^{-1} $year^{-1}$, 321 mg N per seedling) was set to simulate the conditions prevailing in reforested arable lands under Finnish conditions after intensive practice of agriculture.

Fertilizer was dissolved in water and added to the seedlings once a week. The fertilization started in early June and ended in September both in 1999 and 2000. The fertilizers used were applied according to the following regime: Kekkilä SuperX-9 (Kekkilä Oyj, Tuusula, Finland, NPK19:4:20) from June 5 to July 10, Kekkilä SuperX-5 (NPK 12:5:27) from July 17 to August 3, and Kekkilä SuperX-7 (NPK 0:7:32) from August 7 to September 10. All the seedlings were irrigated regularly throughout the experiment. The experimental design and the treatments are described in more detail in Mattson et al. (2004).

2.2. Litter decomposition experiment

At the end of September 2000, after natural abscission, the leaves of similarly treated seedlings within each chamber were collected and pooled. The pooled samples were air dried at room temperature. For the litter decomposition experiment, whole leaves from each pooled unit were divided into a total of 336 litter bags. The size of each bag was 10 cm × 8 cm and they were made of terylene net with mesh size of 1 mm. The mean total dry weight of sample leaves within a bag was 1.9 g (variation between 0.3 g and 4.7 g). The bags were attached side by side in random order into strings. A string consisted of 48 bags, each of them representing a fertilizer and climatic treatment combination within each chamber (i.e. three fertilization treatments × two CO_2 treatments × two temperature treatments × four replications). The length of a string was 4.8 m.

At the end of October 2000, the strings were placed under ambient climate in a naturally regenerated forest on former pasture land near the Mekrijärvi Research station. The litter layer (depth about 5 cm) was removed, and the strings were placed on top of the mineral soil. The strings were buried under detritus of the vegetation in the site. The dominant tree species were Scots pine (*P. sylvestris*) and birch species (*B. pendula* and *Betula pubescens* Ehrh.) at an age of about 30 years with an open canopy. The shrub layer was composed of European rowan (*Sorbus aucuparia* L.) and willows (*Salix* spp.). The field layer was composed of bishop's goutweed (*Aegopodium podagraria* L.), European raspberry (*Rubus idaeus* L.) and different forbs and grasses. The mineral soil under the litter layer contained on average 3–6% of organic matter and was mainly loam.

The litter bags were lifted one string set at a time in May and October in 2001, 2002, and 2003, and in May in 2004. The harvest took place after 214, 376, 601, 763, 971, 1125 and 1357 days from the initiation of the incubation experiment. After each harvest, the remaining leaf litter was taken out of the bags and carefully cleaned from soil and debris. The remaining litter of each bag was dried at room temperature and weighed for mass loss determination.

2.3. Leaf litter quality

The insoluble condensed tannins (mg g^{-1}) and the contents (% DW) of carbon and nitrogen were analyzed before decomposition from the leaf litter in each pooled sample collected. The air dried leaves were crushed and ground to a powder using Pulverisette 6 mill (Fritsh GmbH, Idar-Oberstein, Germany). The condensed tannins were analyzed using the acid–butanol assay (Schofield et al., 1998), and the proportions of C and N using a Leco CHN-1000 elemental analyzer (LECO Corporation, St. Joseph, MI, USA) from three sub-samples from each powdered sample. The sample size in the acid–butanol assay was on average 2 mg DW (variation between 1.4 and 4.7 mg DW), and in the C:N analysis, 150 mg DW.

2.4. Climatic conditions during the litter decomposition experiment

The climatic data was collected at the weather station located 200 m south-east from the experimental site. The mean monthly temperatures (°C) at 10 cm above the ground level did not vary much during the experiment between the years 2001–2004. Within a calendar year, the warmest month on average was July with a mean temperature of 20.1 °C, and the coldest was January with a mean temperature of −11.6 °C.

The monthly precipitation varied slightly between the years. On average, the highest precipitation amount over a calendar year was recorded in July (variation between 47 and 159 mm, the mean value = 84 mm) and the lowest in April (variation between 6 and 47 mm, the mean value = 23 mm). By the time of the first harvest of litter bags, the total cumulative precipitation was approximately 348 mm and the temperature sum approximately 160 °C, and by the time of final harvest, 2690 mm and 3950 °C, respectively. Averaged across the decomposition experiment (four years), the mean precipitation within a year was 585 mm and temperature sum within a growing season 1270 °C. In the calculations for the temperature sum, the base temperature was 5 °C.

2.5. Statistical analyses

The contents of insoluble condensed tannins; carbon and nitrogen; and C:N ratio were tested using mixed model analysis of variance for split-plot design (Linear mixed-effects model from NLME package, R version 2.6.0, The R Foundation for Statistical Computing). Temperature and CO_2, and their interaction were included in the model as fixed terms. Fertilization treatments were nested within the chambers, and included in the model as a fixed covariance term, since the fertilizer was applied to individual plants inside the chambers. The log $(x+1)$ transformation was employed in order to attain the fertilizer levels (0, 150 and 500 kg N ha^{-1} $year^{-1}$) as linear continuous variables. The results are reported as 'significant' if $p \le 0.05$, and 'indicative' if $0.05 \le p \le 0.10$.

To predict the change in remaining litter over time (curves in Fig. 5), a double negative exponential function, described by the following equation, was employed:

$$y = A^* \exp(-k_1{}^*t) + (100 - A)^* \exp(-k_2{}^*t) \quad (1)$$

where "A" is the litter fraction that decays fast and "100 – A" is the fraction that decays slowly, both expressed as percentages. "k_1" and "k_2" are the decay constants for these two fractions. "t" is the time elapsed since the start of the incubation, expressed in days.

The effects of temperature (T), CO_2, and fertilization (F) on the decomposition of silver birch leaf litter (remaining mass, % DW) were studied by fitting the double negative exponential function (Eq. (1)) using a non-linear mixed effect model procedure (nlme function from the NLME package, R version 2.9.0, The R Foundation for Statistical Computing). Temperature and CO_2, and their interaction, and the main effect of fertilization were included in the model as fixed terms, for each of the three parameters of the function. Fertilization treatments were nested within the chambers, since the three fertilizer levels were applied to plants inside each of the chambers. Random effects were included for the three parameters of the function, using chamber as a grouping factor. For the fits k_1 and k_2 were replaced by their exponential. The fitted parameters were the natural logarithms of k_1 and k_2. This was done for computational reasons. The number of replicated units (pooled leaf litter samples) within each climatic and fertilizer treatment combination was four ($n = 4$). Data for $t = 0$ were not used in the fit as they were all 100% by definition, but the equation used forces all curves to 100% remaining mass at $t = 0$. Altogether 3 outliers (in which the contents of the litter bags were less than 10% or more than 80% of the initial dry weight) were removed before statistical analysis. This was due to damage that occurred to the bags during the incubation. The number of bags lost in the field or during retrieval was 12 out of 336 bags. The interaction terms with fertilization were at first included in the model, but the numerical algorithm did not succeed, most probably due to the variability of the data and/or missing observations. For this reason a simpler model had to be fit. The predicted values presented in Fig. 5 were calculated from the output of a variation of the model fit described above, which did not include fertilization as an explanatory factor.

Table 1
Summary of the results in split-plot analysis of variation for the effects of CO_2, temperature, fertilization and their interactions on the insoluble condensed tannins (mg g^{-1}), carbon and nitrogen contents (% DW) and C:N ratio in the leaves at the end of the growing season 2000. The significant values ($p < 0.0.05$) are in bold in the table, and those discussed as indicative ($p < 0.1$) are in italics.

Source	Err df	Tannin content (mg g^{-1})		Carbon (C) content (% DW)		Nitrogen (N) content (% DW)		C:N ratio	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.
CO_2	12	3.24	*0.097*	0.06	0.816	6.10	**0.030**	6.16	**0.029**
Temperature (T)	12	0.00	0.947	2.07	0.175	4.40	*0.058*	2.97	0.110
T × CO_2	12	0.00	0.999	0.32	0.579	0.75	0.405	0.57	0.465
Fertilization (F)	28	5.53	**0.026**	4.28	**0.048**	0.88	0.356	9.74	**0.004**
CO_2 × F	28	0.67	0.421	0.77	0.387	2.82	0.104	5.43	**0.027**
T × F	28	4.08	*0.053*	0.63	0.434	0.02	0.890	0.22	0.643
CO_2 × T × F	28	0.15	0.706	3.38	*0.077*	0.09	0.770	1.57	0.221

3. Results

3.1. Leaf litter quality before incubation

In the leaf litter of birch seedlings, the mean contents of insoluble condensed tannins in different climatic and fertilizer treatment combinations varied between 41.8 mg g^{-1} and 119.5 mg g^{-1} (Fig. 1). The content of tannins increased under elevated CO_2, although the statistical significance of CO_2 treatment was only indicative (Table 1, CO_2, $p = 0.097$, Fig. 1). The main effect of temperature was not statistically significant, but that of fertilization was (Table 1; Fertilization, $p = 0.026$), but there was an indicative interaction between temperature and fertilization treatments (Table 1; Temperature × Fertilization, $p = 0.053$, Fig. 1); the tannin content decreased as nutrient application increased under the ambient temperature but not under the elevated temperature. The largest individual mean in elevated CO_2, ambient temperature and no fertilizer was more than three times larger than the lowest, in ambient temperature and CO_2 with the highest fertilizer dose.

Within the different treatment combinations, the mean contents of carbon (% DW) ranged between 41.6 and 43.8%, and decreased with increased fertilizer application in the elevated temperature treatment and in the elevated CO_2 treatment (Table 1; Fertilization, $p = 0.048$, Fig. 2). However, in both the ambient treatment and elevated temperature plus CO_2, the C content was highest in the moderate fertilization treatment. On average, the highest carbon contents were observed in the leaf litter produced under elevated temperature with no fertilizer application (43.5% DW) and under the combination of elevated temperature and CO_2 with moderate fertilization (43.8% DW) (Table 1; CO_2 × Temperature × Fertilization, $p = 0.077$, Fig. 2).

The mean contents of nitrogen in litters from different climatic and fertilizer treatment combinations varied between 0.7% and 1.6% DW. Elevated CO_2 decreased the N content (Table 1; CO_2, $p = 0.030$, Fig. 3); the content was about 1.3% DW under ambient CO_2, whereas under elevated CO_2 it was about one-fourth less, approximately 0.9%. The highest content of N was in the litter produced under elevated temperature, 1.5% DW, although the effect of temperature treatment was only indicative (Table 1; Temperature, $p = 0.058$, Fig. 3). The fertilization treatment itself caused no statistically significant effect on N content.

The mean values of C:N ratio in the different climatic and fertilizer treatment combinations varied between 28 and 63. In contrast to the N content, the C:N ratio was significantly decreased by increased fertilizer application (Table 1; Fertilization, $p = 0.004$, Fig. 4), but not affected by temperature treatment. Instead, C:N ratio

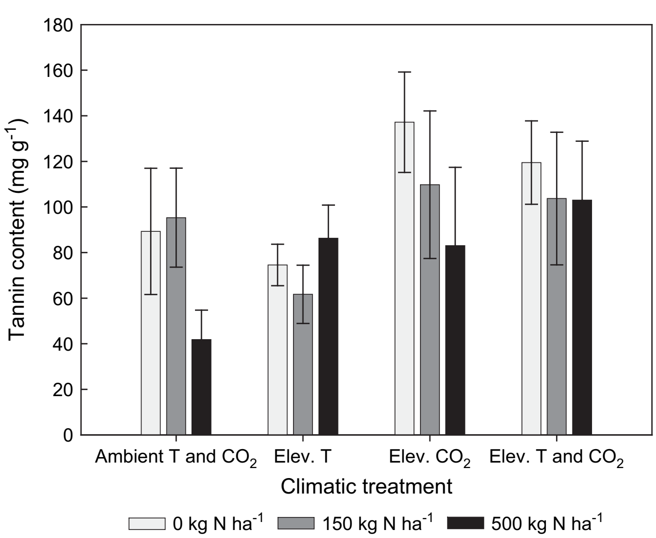

Fig. 1. Content of insoluble condensed tannins (mg g^{-1} DW, +SE bars) in the birch leaves within the different fertilizer (0, 150 and 500 kg N ha^{-1}) and climatic treatment combinations (combination of ambient temperature and CO_2, elevated temperature, elevated CO_2 and combination of elevated temperature and CO_2) before leaf litter decomposition experiment ($n = 4$).

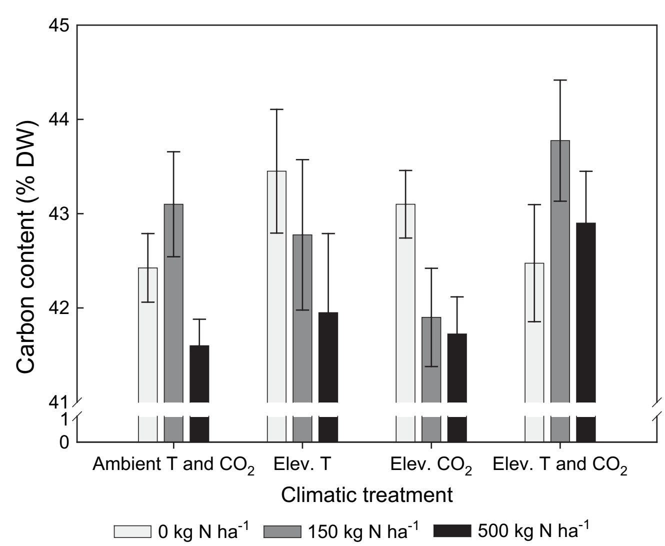

Fig. 2. Proportion of carbon (% DW, +SE bars) in the birch leaves within the different fertilizer and climatic treatment combinations before leaf litter decomposition experiment ($n = 4$).

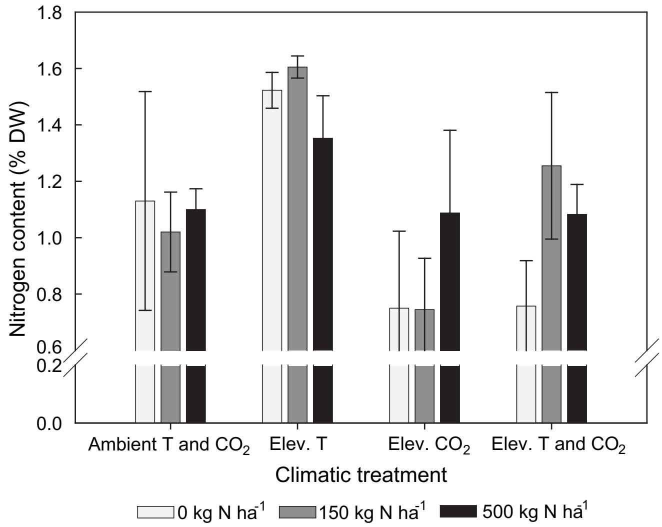

Fig. 3. Proportion of nitrogen (% DW, +SE bars) in the birch leaves within the different fertilizer and climatic treatment combinations before leaf litter decomposition experiment ($n = 4$).

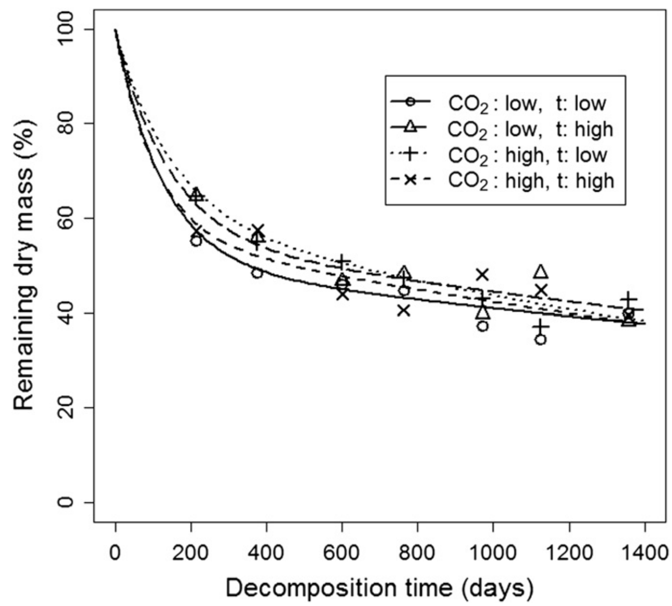

Fig. 5. Remaining leaf litter mass (% DW) vs. incubation time (days). The symbols represent the measured median values across the fertilizer levels within the climatic treatments and the curves the predicted remaining litter over time from an NLME model fit to Eq. (1). Fertilization not included as explanatory factor for simplicity. See text for details.

was dependent on CO_2 treatment, and the interaction of fertilization and CO_2 was significant (Table 1; CO_2, $p = 0.029$; $CO_2 \times$ Fertilization, $p = 0.027$, Fig. 4). The ratio was higher in the leaves produced under elevated CO_2. However, under elevated CO_2, the ratio decreased significantly as nutrition increased (Table 1). Under ambient CO_2, the C:N ratio was lower, and the fertilizer treatment did not significantly affect it (Fig. 4).

3.2. Leaf litter decomposition

Averaged across the fertilizer levels, the remaining mass of litter produced under the different climatic treatments varied between 56% and 65% DW at the time of the first harvest in May 2001 (Fig. 5). At the end of the experiment, it varied between 38% and 47%.

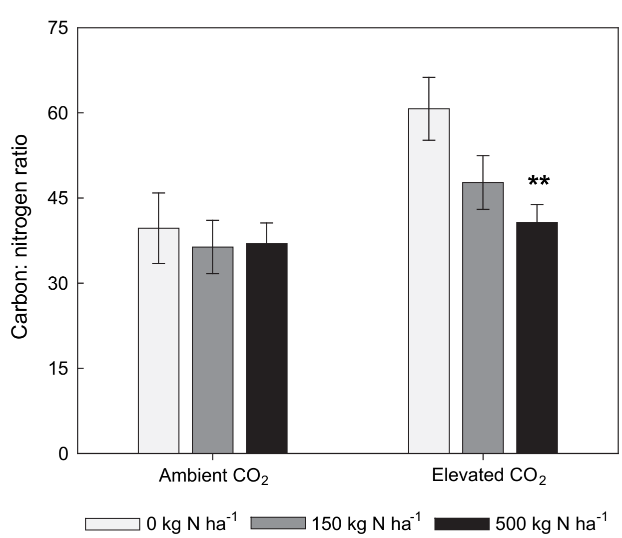

Fig. 4. Carbon: nitrogen ratio (+SE bars) in the birch leaves within the different CO_2 treatment levels before leaf litter decomposition experiment ($n = 4$). The symbol '**' indicates statistically significant ($p < 0.01$) difference in C:N ratio of the leaves between low N treatment (unfertilized seedlings) and high N treatment (500 kg N ha^{-1}) (Bonferroni adjusted pair-wise comparisons of estimated marginal means).

The fitted model describes the data well (Fig. 5). However, in a non-linear mixed-effects model, calculations making use of degrees of freedom are broad approximations and not very meaningful. The most commonly held opinion amongst statisticians is that given the current state of the art, p values should not be calculated or used for decision making with this kind of model. For this reason we only present F-values in Table 2. Nonetheless, effects with large F-ratios (arbitrarily taken as values larger than 50) are considered worth discussing.

The initial percentage of the "fast" decaying litter fraction (A) was affected by CO_2 and temperature elevation with interaction, and also by fertilization, varying between 35 and 57% (Fig. 6). The "fast" fraction was smallest in litter from seedlings grown at elevated CO_2 and temperature, and it was largest under ambient conditions. There was also an increase of the size of this fraction in response to fertilization, especially at the highest dose.

The decay constant for the "fast" litter fraction (k_1 in Eq. (1)) was markedly affected by all treatments, its value varying between 0.0049 and 0.0185 d^{-1} equivalent to half lives of 141 and 37 days respectively (Fig. 7). Elevated temperature during seedling growth slowed down decomposition under ambient CO_2, but markedly increased its speed under elevated CO_2. Fertilization had a marginal positive effect at 150 kg ha^{-1} but markedly slowed down the decay rate of the "fast" litter fraction at the 500 kg ha^{-1} dose.

The decay constant for the "slow" litter fraction (k_2 in Eq. (1)) was not affected by the treatments and its mean estimated value

Table 2
F-ratios from NLME analysis. Approximate degrees of freedom 1 and 287, except for fertilization for which they are 2 and 287. From model fit to Eq. (1) to remaining litter dry mass values measured at 214, 376, 601, 763, 971, 1125 and 1357 days of incubation.

Treatment	A	k_1	k_2
CO_2	176	229	6.29
T	16.9	6.09	0.16
$CO_2 \times$ T	68.8	188	1.34
Fertilization	365	439	0.18

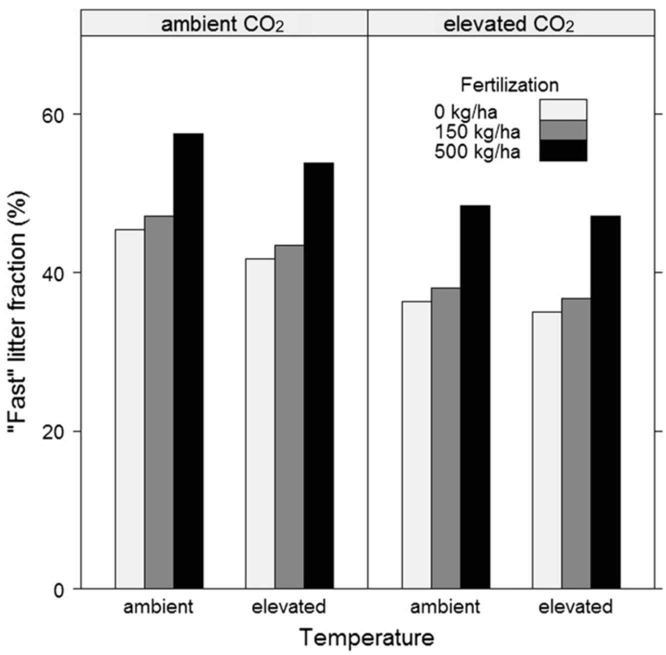

Fig. 6. "Fast" decomposing litter fraction as a percent of total litter dry mass, in fresh silver birch leaf litter (at day = 0 of incubation period). Estimates from model fit to double negative exponential function (Eq. (1)).

was 0.000292 d^{-1} that represents a half-life of 2370 days. However, the range of estimated values across treatments spanned from 0.000105 to 0.000467 d^{-1}.

4. Discussion

The objective of this study was to determine if the quality of silver birch leaf litter is altered by single and concurrent increases in CO_2, temperature and nutrient availability, and if this alteration is reflected in the decay rates of abscised leaves. Therefore, litter produced under the different treatment combinations was left to be decomposed in the field under natural conditions, which made it possible to compare the rate of decomposition of litters with different quality over time.

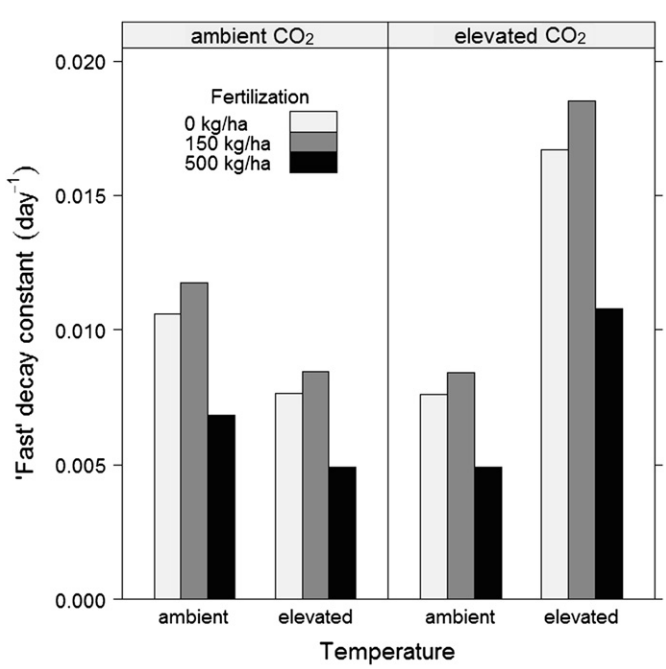

Fig. 7. Decay constant for "fast" litter fraction (k_1 in Eq. (1)) in silver birch leaf litter. Estimates from model fit to double negative exponential function (Eq. (1)).

The estimated proportion of the fast-decomposing fraction (A in Eq. (1)) was smallest in litter from seedlings grown at elevated CO_2 and temperature, and it was largest under ambient conditions. The decay constant for the "fast" litter fraction (k_1 in Eq. (1)) was markedly affected by all treatments (Fig. 7). Elevated temperature during seedling growth slowed down decomposition of the "fast" decomposing fraction of the litter from ambient CO_2, but markedly increased decomposition speed in litter from elevated CO_2. There was an increase in the estimated proportion of the fast-decomposing fraction (A) in response to fertilization, especially at the highest dose (500 kg ha^{-1}). However, fertilization also markedly slowed down the decay rate of the "fast" litter fraction at the dose in all CO_2 and temperature combinations. The fastest decay rate was found in the two lower fertilizer levels in both elevated temperature and CO_2, which also had the smallest fraction of the fast-decomposable litter. It cannot be completely ruled out that these compensating effects on the size of the "fast" fraction and its decay rate are an "artifact" of the curve fitting process as these parameters were negatively correlated ($r = -0.65$ to -0.75 depending on the treatment effect considered), but at least in part could be related to changes in litter.

The treatment differences in the amount of tannins, carbon and nitrogen could have been expected to affect the initial processes. Earlier studies have reported that the disappearance of tannins is rather quick from the leaf litter. For example, Schofield et al. (1998) found that the tannins in sandbar willow (*Salix exigua* Nutt.) were essentially gone by six weeks after the leaf fall. Therefore, it is unlikely that the initial contents of tannins would explain the time-dependent differences in mass loss during the whole decomposition experiment, but they might have affected the early stages of the process. Tannins are secondary polyphenols of high molecular weight with bitter taste and an ability to precipitate proteins and thereby slow the consumption of litter by the decomposers (Harrison, 1971; Bryant et al., 1992). However, here, the lower amount of tannins in the litter produced at the higher fertilizer levels (only in ambient T) does not explain the reduced early-stage decay rate at the high fertilizer levels. Moreover, the higher amount of tannins in elevated CO_2 did not explain the initial decay rates of the fast-decomposing fraction as such, as the decay of the elevated CO_2-litter depended on the temperature regime.

Litter decomposition rates may often be more readily explained by the relations of C and N than each of them separately. Here, there was a three-way interaction on the C content, which was reduced by fertilization in abscised leaves produced either under elevated temperature or elevated CO_2; however, in the extreme treatments the intermediate fertilizer level had the highest C levels (Fig. 2). There was no clear effect of CO_2 elevation on C content, although earlier studies have shown elevated CO_2 has resulted in increases in the contents of lignin and cellulose in the leaves of many plant species (Cotrufo et al., 1994; Cotrufo and Ineson, 1996; Ziska et al., 2005; Kasurinen et al., 2006, 2007). In this study, however, it was unfortunately not possible to measure lignin and cellulose contents. Commonly, these polymer carbohydrates are the most abundant elements in leaves (Fioretto et al., 2005), and they may have composed the main part of the total carbon in the silver birch litter. The effects of elevated temperature have been studied much less, but there are results on increases in lignin, hemicellulose and cellulose in the leaves of some temperate grasses (Ford et al., 1979).

Fioretto et al. (2005) found in the litter of holly oak (*Quercus ilex* L.), that cellulose was almost completely degraded in 27 months, but lignin did not show any significant degradation during that period. Lignin is considered to degrade mostly at the later stages of the process (Suberkropp et al., 1976; Choong et al., 1992; Gallardo and Merino, 1993; Wardle et al., 1998; Pérez-Harguindeguy et al., 2000). Moreover, high amounts of lignin may interfere with the enzymatic degradation of carbohydrates (such as cellulose and hemicellulose) and proteins (Cooke and Whipps, 1993), and therefore slow down the rates of decomposition (Melillo et al., 1982). Possible alterations in lignin degradation would be mostly related to changes in k_2 in our study, and these could have remained undetected because of short incubation time in relation to half-life of the slow fraction. Estimates for k_2 varied considerably in value suggesting a lack of sensitivity of our experiment for effects on this parameter.

The total nitrogen in the abscised birch leaves was decreased by CO_2 and increased by temperature, but not significantly affected by fertilization with complete fertilizers. The increase in the initial N content by elevated temperature was large, as could be expected, considering the enhancing effects of a slightly higher temperature on physiological and microbiological processes in the temperature ranges of a boreal-region summer. The temperature effect was not so large at the highest fertilization treatments, as the importance of soil processes would be smaller at high external nutrient availability; however there was no significant interaction on N content. Temperature did not affect the C:N ratio, as it tended to increase both C and N. By contrast, the N content in the litter was substantially decreased by the elevation of CO_2 and, accordingly, the C:N ratio was higher than that under ambient CO_2. This result is consistent with previous findings in leaves prior to their abscission in autumn, where the exposure to elevated CO_2 has lowered the N content (Cotrufo et al., 1994; Kasurinen et al., 2006) and increased the C content (Cotrufo et al., 1994; Kasurinen et al., 2006), thereby leading to an increase in the C:N ratio.

The lack of statistical significance of the fertilization treatment on N content is partly due to the large variability, but it may be due to the higher growth rate of fertilized seedlings, in which case nitrogen has been allocated to the production of new tissues during the growing season (Mattson et al., 2004). Also, regardless of nutrient availability, proteins and chlorophyll are degraded and transported from senescing leaves to other plant parts (Himelblau and Amasino, 2001). Therefore, the content of N in abscised leaves is much smaller compared to the maximum in mid-summer (Chapin and Kedrowski, 1983), and treatment differences are probably not the same as they would be during the growing season. Sometimes there is no effect of N fertilization on the N content of abscising leaves or needles (T. Lehto, P.J. Aphalo, P. Saranpää, T. Laakso and A. Smolander, unpublished).

It has been shown that higher nitrogen contents in litter often stimulate the initial stages of decomposition, although at later stages high N can retard the decomposition rates (e.g. Rosswall, 1982; Hodge et al., 2000). Nevertheless, the initial N content alone did not directly explain the mass loss of silver birch litter in the present study, and neither did the C:N ratio, as the treatment differences in decomposition were not consistent with either. Longer-term effects of the "slow" fraction will be of interest in further studies, as its proportion was increased in the elevated CO_2 and temperature regime, but within all treatments, decreased by fertilization.

In this experiment, the initial quality and decomposition rate of the silver birch litter was substantially changed by individual and particularly, interactive effects of the three factors studied. In an actual climate with increasing temperatures also during decomposition, the effects of CO_2 and temperature during growth might counteract a possibly increasing decomposition rate due to higher soil temperature during decomposition. The changes found in the litter decomposition rates in this study were not very large. However, their importance on the long term will depend on whether they are consistent among plant species and various environmental conditions. The study highlights the importance of studying several environmental factors together. As the interactive effects can be different from those of each individual factor, the predictions based on single-factor experiments may be altered if different factors are taken into account.

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