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Chemical composition and decomposition of silver birch leaf litter produced under elevated CO_2 and O_3

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Abstract

Two field-growing silver birch (*Betula pendula* Roth) clones (clone 4 and 80) were exposed to elevated CO_2 and O_3 over three growing seasons (1999–2001). In each year, the nutrients and cell wall chemistry of naturally abscised leaf litter were analyzed in order to determine the possible CO_2- and O_3-induced changes in the litter quality. Also CO_2 and O_3 effects on the early leaf litter decomposition dynamics (i.e. decomposition before the lignin decay has started) were studied with litter-bag experiments (Incubation 1 with 1999 leaf litter, Incubation 2 with 2000 leaf litter, and Incubation 3 with 2001 leaf litter) in a nearby silver birch forest. Elevated CO_2 decreased N, S, C:P and α-cellulose concentrations, but increased P, hemicellulose and lignin + polyphenolic concentrations, C:N and lignin + polyphenolic:N in both clones. CO_2 enrichment decreased the subsequent decomposition of leaves of clone 4 transiently (in Incubations 1 and 2), whereas elevated CO_2 effects on the subsequent leaf decomposition of clone 80 were inconsistent. In contrast to CO_2, O_3 decreased P concentrations and increased C:P, but both of these trends were visible in elevated O_3 treatment only. O_3-induced decreases in Mn, Zn and B concentrations were observed also, but O_3 effects on the cell wall chemistry of leaf litter were minor. Some O_3-induced changes either became more consistent in leaf litter collected during 2001 (decrease in B concentrations) or appeared only in this litter lot (decrease in N concentrations, decrease in decomposition at the end of Incubation 3). In conclusion, in northern birch forests elevated CO_2 and O_3 levels have the potential to affect leaf litter quality, but consistent CO_2 and O_3 effects on the decomposition process remain to be validated.

Introduction

Background concentrations of CO_2 and ground-level O_3 are increasing globally (IPCC, 2001). Both increased CO_2 and O_3 levels are known to affect tree growth and carbon allocation severely, CO_2 usually enhancing and O_3 decreasing tree productivity (Andersen, 2003; Ceulemans and Mousseau, 1994; Cooley and Manning, 1987; Pye, 1988; Saxe et al., 1998; Norby et al., 1999; Karnosky et al., 2003). Several studies indicate that the chemical composition of green leaves (e.g. N concentrations, C:N, total sugars, phenolic compounds, lignin and cellulose concentrations) can change due to increased CO_2 (Blaschke et al., 2002; Gifford et al., 2000; Kuokkanen et al., 2001; Poorter et al., 1997) and O_3 levels

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(Cabané et al., 2004; Cooley and Manning, 1987; Landolt et al., 1997; Saleem et al., 2001; Yamaji et al., 2003). In addition, it has been also assumed that elevated CO_2 and O_3 can alter the leaf litter decomposition rates through CO_2- and O_3-induced changes in leaf litter chemical composition (Andersen, 2001; Cotrufo et al., 1994).

If CO_2- and O_3-induced changes in the chemical composition of green leaves persist throughout the leaf senescence, this could have a severe impact on nutrient cycling as well as organic soil formation within the forest ecosystems, as both of these processes are governed by leaf litter quality and decomposition rates (Cadisch and Giller, 1996; Swift et al., 1979). The current evidence of CO_2- and O_3-induced changes in leaf litter quality and decomposition is contradictory (Andersen, 2001; Boerner and Rebbeck, 1995; Coûteaux et al., 1999; Findlay and Jones, 1990; Gifford et al., 2000; Norby and Cotrufo, 1998; Norby et al., 2001; O'Neill and Norby, 1996). As observed in earlier studies, CO_2 effects are most often visible in leaf litter C:N and N concentrations (Cotrufo et al., 1998c; Coûteaux et al., 1999), as tree leaf litter produced under elevated CO_2 usually has higher C:N and lower N concentrations than that produced under ambient CO_2 conditions (Coûteaux et al., 1996, 1999; O'Neill and Norby 1996). Lignin concentrations can be affected by elevated CO_2 as well, but lignin response to CO_2 is highly variable and is known to vary from positive to negative in trees (Cotrufo and Ineson, 1996; Coûteaux et al., 1996, 1999). Similarly, CO_2 can either decrease or increase subsequent decomposition rates of leaf litter, but in general the decomposition of leaves exposed to increased CO_2 levels during their growth period has not been observed to change significantly (Boerner and Rebbeck, 1995; Cotrufo et al., 1994, 1998b; Coûteaux et al., 1996, 1999; King et al., 2001).

O_3 effects on leaf litter quality are less studied than those of elevated CO_2, but there is some indication that elevated O_3 could modify lignin concentrations and also change N release during the decomposition (Boerner and Rebbeck 1995). As with CO_2 (O'Neill and Norby 1996), the most obvious O_3-induced decreases in subsequent leaf litter decomposition rates have been observed in laboratory incubations (Findlay and Jones, 1990; Findlay et al., 1996), whereas in field incubations the O_3 effects on the subsequent decomposition have been usually negligible (Boerner and Rebbeck, 1995; Kainulainen et al., 2003). The knowledge about CO_2 and O_3 interaction effects on leaf litter chemical composition and decomposition is even more scarce. In a long-term field study, no elevated CO_2 and O_3 interaction effects on Scots pine needle litter chemistry (resin acids, terpenes and total phenolics) and thereby on decomposition were found (Kainulainen et al., 2003). However, Scherzer et al. (1998) observed that yellow-poplar leaf litter grown under $CO_2 + O_3$ treatment had consistently lowest N concentrations and it also decomposed slowest. Since Scherzer et al. (1998) observed that O_3 fumigation under ambient CO_2 did not affect yellow-poplar leaf litter significantly, they suggested that this negative effect of CO_2 and O_3 combination treatment on yellow-poplar leaf litter chemical composition and subsequent decomposition was due to CO_2 alone. More support for this suggestion comes from another study, where CO_2 enrichment altered paper birch leaf litter quality and decreased subsequent decomposition regardless of prevailing O_3 levels (Parsons et al., 2004).

In the present study, two silver birch (*Betula pendula* Roth) clones (field-growing trees) were exposed to elevated CO_2 and O_3, alone and in combination, in open-top chambers for three consecutive growing seasons (1999–2001). The main aim of this study was to investigate whether the chemical composition of silver birch leaf litter and the early stages of subsequent decomposition (i.e. decomposition before the lignin decay has started) are changed due to three-year CO_2 and O_3 exposures. The leaf litter produced in the chamber treatments was collected during maximum leaf abscission in each year, and single and interaction effects of CO_2 and O_3 on leaf litter nutrients, cell wall chemistry and subsequent decomposition were studied. Our assumptions were that elevated CO_2 could lead to poorer quality leaf litter (e.g. increase in C:N and decrease in nutrients) and that these CO_2-induced changes in leaf litter quality could be manifested in the subsequent leaf litter decomposition (i.e. slow down the early stages of decomposition) as well. In addition, we suggested that elevated CO_2 effects on leaf litter quality and decomposition would be more obvious than those of elevated O_3, and also that elevated CO_2 in combination with elevated O_3 would affect leaf litter chemical

composition and decomposition in a similar manner as elevated CO_2 alone.

Materials and methods

Open-top chamber experiment: experimental site and design

The present open-top chamber experiment was conducted at a field site located near Suonenjoki Research Station of the Finnish Forest Research Institute (62°39′ N, 27°03′ E, 120 m asl) in central Finland. In this open-top chamber (OTC) experiment, two silver birch genotypes (7-year-old, field-growing clones 4 and 80) originating from southern part of Finland were exposed to elevated CO_2 and O_3, alone and in combination, for three consecutive growing seasons (1999–2001). Of the 15 clones growing at the site, these two clones were selected for the present OTC experiment on the basis of their differing O_3-sensitivity. In a previous survey, clone 4 had been classified as an O_3-tolerant and clone 80 as an O_3-sensitive genotype (Pääkkönen et al., 1997). In total, 32 trees were grown inside open-top chambers and trees were randomly divided into the following treatment groups: (1) ambient CO_2 and ambient O_3, chamber control (CC) (2) $2\times$ ambient CO_2 and ambient O_3 ([CO_2] approximately 720 ppm) (EC), (3) ambient CO_2 and $2\times$ ambient O_3 (EO) and (4) $2\times$ ambient CO_2 plus $2\times$ ambient O_3 (EC + EO). Each treatment was replicated four times for each clone, and each chamber (6 m tall $\times$ 2.5 m wide in 1999 and 7.8 m tall $\times$ 2.5 m wide in 2000–2001) contained one experimental tree (Vapaavuori et al., 2002).

In the first experimental year 1999, CO_2 and O_3 fumigations lasted from late May to the beginning of October, whereas in years 2000 and 2001 exposures were run from early May to the end of September. Exposure time for CO_2 was 24 h day^{-1} during the whole experiment, while O_3 fumigations were run for 12 h day^{-1} in 1999 and 2000 (between 0800 and 2000 h) and 14 h day^{-1} (between 0800 and 2200 h) in 2001. At the Suonenjoki site, the daily mean concentration of O_3 concentrations varied from 10 to 60 ppb during the three growing seasons. The AOT40 values for elevated O_3 were 20–30 ppm · h at the end of each exposure season meaning that the critical threshold value for trees (10 ppm · h) was exceeded during each experimental season. There were no differences in soil fertility conditions between treatments and clones, and the concentrations of main nutrients were similar to those previously reported from nursery and forest soils (Kasurinen, et al., 2004, Luoranen, 2000, Vapaavuori et al., 2002). For further information about the experimental field and soil, clones, open-top chambers and exposure system, see Kasurinen et al. (2004), Mutikainen et al. (2000) and Vapaavuori et al. (2002).

Chemical analyses of leaf material

The concentrations of P, K, Ca, Mg, Mn, Fe, Zn, Cu, B and S in leaf litter and green leaves were determined according to method introduced by Halonen et al. (1983) and using plasma emission spectrophotometry analysis (ICP, ARL 3000), while total C and N concentrations of leaves were measured with a CHN-1000 Analyzer (Leco Co., St. Joseph, MI, USA). Naturally abscised leaf litter was collected weekly from the ground of the open-top chambers (CC, EC, EO and EC + EO-trees) from July onward during the exposure periods of 1999–2001. Each leaf litter lot was cleaned from soil and debris and air-dried at room temperature before any further analyses. Leaves used in the chemical analyses and litter-bag studies were taken from same leaf litter lots collected in periods 11.9.–5.10.1999, 23.9.–13.10.2000 and 10.9.–1.10.2001 (e.g. during the maximum autumnal leaf abscission periods). For the C and N analyses green leaves still attached to short- and long-shoots were collected on 24th of August 1999, 25th of August 2000 and 5th of September 2001, respectively, while leaf samples for P, K, Ca, Mg, Mn, Fe, Zn, Cu, B and S analyses were taken on 7th of September during each study year. Clone, CO_2 and O_3 effects on nutrient concentrations in green leaves are shown for reference in Table 1. The data obtained from separate short- and long-shoot leaves was pooled together in order to obtain tree means ($n = 4$ per treatment).

In order to study leaf litter cell wall chemistry, milled leaf sample (0.5 g) was extracted in acetone (150 ml) by Soxhlet method for gravimetric

Table 1. Nutrient concentrations, C:N and C:P status of green leaves in 1999–2001, when short- and long-shoot leaves were pooled together (CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO elevated CO_2 and O_3 in combination; $n = 4$ per treatment).

Treatment	Clone	CC	EC	EO	EC + EO
N (mg g^{-1})	4	21.15 ± 0.50	18.86 ± 0.70	19.41 ± 0.58	18.64 ± 0.67
	80	21.59 ± 0.19	17.91 ± 0.28	20.48 ± 0.62	18.99 ± 0.61
P (mg g^{-1})	4	3.73 ± 0.30	4.08 ± 0.12	3.33 ± 0.24	4.01 ± 0.24
	80	4.41 ± 0.09	4.91 ± 0.32	3.71 ± 0.27	4.42 ± 0.49
K (mg g^{-1})	4	9.40 ± 0.47	8.65 ± 0.46	9.01 ± 0.40	8.84 ± 0.42
	80	8.49 ± 0.46	7.62 ± 0.54	9.44 ± 0.28	7.65 ± 0.57
Ca (mg g^{-1})	4	6.27 ± 0.17	5.52 ± 0.09	5.36 ± 0.26	5.67 ± 0.20
	80	8.54 ± 0.69	8.89 ± 0.52	7.36 ± 0.83	8.66 ± 1.32
Mg (mg g^{-1})	4	2.53 ± 0.16	2.18 ± 0.12	2.28 ± 0.07	2.27 ± 0.13
	80	2.89 ± 0.21	3.13 ± 0.21	2.69 ± 0.20	2.92 ± 0.24
Mn (mg g^{-1})	4	1.09 ± 0.17	1.15 ± 0.08	0.76 ± 0.15	0.85 ± 0.15
	80	1.12 ± 0.12	1.33 ± 0.08	0.96 ± 0.20	1.03 ± 0.16
Fe (μg g^{-1})	4	54.19 ± 1.42	48.46 ± 1.66	46.84 ± 1.18	48.46 ± 3.93
	80	60.47 ± 1.25	56.10 ± 2.29	56.65 ± 2.93	57.63 ± 4.66
Zn (μg g^{-1})	4	302.1 ± 24.9	346.3 ± 32.4	261.7 ± 24.0	282.3 ± 9.2
	80	365.7 ± 35.8	385.2 ± 47.9	300.9 ± 27.3	315.8 ± 15.6
Cu (μg g^{-1})	4	5.44 ± 0.41	4.29 ± 0.25	4.78 ± 0.30	4.27 ± 0.18
	80	5.12 ± 0.47	4.51 ± 0.44	4.63 ± 0.20	4.47 ± 0.15
B (μg g^{-1})	4	41.16 ± 3.74	44.75 ± 2.38	39.89 ± 2.11	35.30 ± 2.57
	80	58.33 ± 3.35	53.42 ± 6.55	51.31 ± 2.95	46.15 ± 4.08
S (μg g^{-1})	4	1437.5 ± 36.2	1198.6 ± 34.1	1290.0 ± 38.5	1204.5 ± 61.7
	80	1323.8 ± 40.6	1168.9 ± 27.4	1238.6 ± 23.2	1133.3 ± 41.3
C:N	4	23.98 ± 0.58	26.84 ± 1.02	26.51 ± 0.77	27.12 ± 0.99
	80	23.41 ± 0.35	27.70 ± 0.55	25.15 ± 0.86	26.50 ± 1.12
C:P	4	138.3 ± 12.4	123.1 ± 3.8	156.6 ± 12.0	126.3 ± 8.7
	80	115.7 ± 2.4	99.1 ± 6.4	137.9 ± 10.2	117.1 ± 16.1
Variable	Clone		CO_2	O_3	$CO_2 \times O_3$
N	[a]		< 0.0005[b]	[a]	0.029[e]
P	0.009	4 < 80	0.010[c]	0.051[d]	[a]
K	0.047	4 > 80	0.011[b]	[a]	[a]
Ca	< 0.0005	4 < 80	[a]	[a]	[a]
Mg	< 0.0005	4 < 80	[a]	[a]	[a]
Mn	[a]		[a]	0.014[b]	[a]
Fe	< 0.0005	4 < 80	[a]	[a]	[a]
Zn	0.045	4 < 80	[a]	0.008[b]	[a]
Cu	[a]		0.013[b]	[a]	[a]
B	< 0.0005	4 < 80	[a]	0.025[b]	[a]
S	0.025	4 > 80	< 0.0005[b]	0.027[b]	0.081[e]
C:N	[a]		0.001[c]	[a]	0.040[e]
C:P	0.017	4 > 80	0.009[b]	0.045[c]	[a]

Values are mean ± SE over a three-year period (units mg g^{-1} or μg g^{-1} of leaf d wt)
p-values are from the between-subjects effects test in RM-ANOVA.
[a] $p > 0.1$; Significant [b]decrease, [c]increase, [d]marginally significant decrease, [e]EC≈EC + EO and CO_2 effect > O_3 effect.

measurement of acetone-soluble extractives and for gaining extractive-free samples (SCAN-AM, 1994) of which then total lignin (gravimetric lignin plus acid-soluble lignin), α-cellulose and hemicellulose (important cell wall component in deciduous trees) fractions as well as total sugars (i.e. the total amount of carbon in the structural carbohydrates of cell wall expressed as glucose units) and uronic

acids (main component of hemicellulose) were analyzed thereafter (see Anttonen et al., 2002 and references therein for further information). However, the leaf material of deciduous trees contains large amounts of other polyphenolics and suberin as well and these fractions form insoluble complexes with lignin. Therefore, the total lignin concentration represented here is actually the sum of total lignin and other polyphenolics (hereafter referred to as lignin + polyphenolic fraction). Finally, C:N, C:P (for both leaf litter and green leaf samples) and lignin + polyphenolic:N were calculated as well. All chemical analyses of leaf litter were performed with leaves collected from individual chambers ($n = 4$ per treatment).

Litter-bag experiments (Incubations 1–3)

Three separate litter-bag incubation experiments were conducted with the leaf litter collected during the 1999 (Incubation 1), 2000 (Incubation 2) and 2001 (Incubation 3) maximum leaf abscission periods. All of these leaf litter incubations were conducted in the silver birch forest located near the OTC field of Suonenjoki Research Station. Since the leaf litter taken from four replicate trees per treatment was pooled together before weighing it into the litter-bags, the actual unit of replication is the litter-bag instead of the individual tree in each litter-bag Incubation experiment. Thus, in the first litter-bag study (Incubation 1) altogether 80 ($n = 10$ bags per treatment) mesh bags (26 cm × 12 cm) constructed of 1 mm aperture fiberglass cloth were buried below the litter layer in the top soil, whereas in the second (Incubation 2) and third study (Incubation 3) the total number of litter-bags was 160 ($n = 20$ bags per treatment). Nevertheless, as some of the litter-bags were omitted from the statistical analyses due to handling errors (e.g. leaves could not be cleaned without crushing them), the actual number of replicates was 7–9 in Incubation 1 and 18–20 in Incubations 2 and 3 (i.e. 3–5 bags per harvest in Incubation 1, and 5–8 bags per harvest in Incubations 2 and 3).

At the beginning of the each incubation each litter-bag contained approximately 2.5 g of air-dried leaf litter. In Incubation 1 conducted with year 1999 leaf litter litter-bags were harvested twice (i.e. the first set of litter-bags was collected after 255 days and the rest after 318 days), whereas in Incubation 2 (year 2000 leaf litter) and Incubation 3 (year 2001 leaf litter), bags were harvested three times (after 259, 285 and 320 days in Incubation 2, and after 263, 291 and 326 days in Incubation 3). After harvesting the litter-bags were immediately taken to the laboratory, and leaves taken inside the bags were cleaned of soil and debris within 72 h. After cleansing, leaf litter was air-dried and the remaining mass of leaf litter was determined.

Statistical analysis

The nutrient concentrations of green leaves and leaf litter as well as the cell wall chemistry of leaf litter were tested with repeated measures ANOVA (RM-ANOVA; fixed factors CO_2, O_3, clone and year), whereas the data from litter-bag incubations was tested with four-way ANOVA (4-way ANOVA; fixed factors CO_2, O_3, clone and harvest day). Before the analyses, all variables were checked for the normality and homogeneity of variances and when necessary, variables were transformed in order to meet the assumptions of the ANOVA tests (log-, square root and arcsin-transformations were employed). In addition, the structure of relationship (e.g. potential covariation) between different leaf litter nutrient and cell wall chemistry parameters were studied with Principal Component Analysis (PCA) employing Varimax Rotation, and then the correlations between different leaf litter quality components formed with PCA and the remaining leaf litter mass % were studied with Pearson's correlation analysis. Since the replication unit in the leaf litter quality data was the individual tree and in the decomposition data the individual litter-bag, the treatment means for all parameters were calculated and used in the PCA analysis and Pearson's correlation analyses ($n = 8$ in both tests). All statistical analyses were conducted with SPSS 11.5 for Windows. Differences were considered statistically significant when p-value ≤ 0.05 and marginally significant when p-value ≤ 0.1.

Results

CO_2 and O_3 effects on leaf litter nutrients

In general, elevated CO_2 treatments decreased N, K and S concentrations and increased P concentrations in both clones (Table 2). In clone 4, decrease in N and S concentrations due to CO_2 was most apparent in leaf litter collected during 1999, while in clone 80 the adverse CO_2 effects became stronger towards the end of the OTC experiment (year×clone×CO_2 effects, $p = 0.013$ for both N and S, Table 3). The CO_2-induced decrease in K concentrations (Table 2) was mainly due to the negative response of clone 80 leaf litter to elevated CO_2 treatments in 1999 and 2001, whereas in clone 4 the CO_2 effects on leaf litter K levels remained negligible during these three years (year×clone×CO_2 effect, $p = 0.001$, Table 3).

Elevated O_3 decreased leaf litter P concentrations, but in both clones this trend was observed in EO treatment only (Table 2). In addition to P concentration, elevated O_3 decreased leaf litter B, Mn and Zn concentrations as well (Table 2). A negative O_3 effect on leaf litter N was observed in 2001, as elevated O_3 decreased N concentrations in both clones (year×O_3 effect, $p = 0.045$, Table 3). The O_3-caused decrease in leaf litter B concentrations had also a tendency to become more consistent towards the end of the OTC experiment in both clones (year×O_3 effect, $p = 0.072$, Table 3). In clone 4 elevated O_3 treatments decreased Mn concentrations in each year, whereas in clone 80 this negative O_3 effect gradually appeared as the OTC experiment progressed (year×clone×CO_2×O_3 effect, $p = 0.021$, Table 3).

CO_2 and O_3 effects on leaf litter cell wall chemistry

Elevated CO_2 decreased α-cellulose concentration, but increased hemicellulose, total lignin + polyphenolic and gravimetric lignin concentration in the leaf litter of both clones (Table 4). In clone 80 both CO_2 treatments (EC and EC + EO) decreased the amount of acetone-soluble extractives whereas in clone 4 the effects of CO_2 were negligible (Table 4). Elevated CO_2 also tended to decrease leaf litter uronic acid concentration in clone 4 and increase it in clone 80 (Table 4), this clonal difference in CO_2 response becoming more apparent towards the end of the OTC experiment (year×clone×CO_2 effect, $p = 0.040$, Table 5).

Statistically significant CO_2×O_3- and marginally significant clone×CO_2×O_3-interaction effect revealed that in both clones EC and EO treatments increased acid-soluble lignin concentration, but the response to EC + EO differed between these two clones, as in clone 4 EC + EO treatment increased and in clone 80 decreased acid-soluble lignin concentration in leaf litter (Table 4). However, in 2001, the increase of acid-soluble lignin due to EC and EC + EO treatments became apparent in both clones over time (year×CO_2 effect, $p < 0.0005$, Table 5), although this increase was somewhat larger in clone 80 than in clone 4 leaf litter (year×clone×CO_2 effect, $p = 0.061$, Table 5). Finally, the direction of uronic acid O_3 response changed significantly over time and depended on the tree genotype as well (year×clone×O_3 effect, $p = 0.042$, Table 5). Thus, O_3 decreased leaf uronic acids concentration in clone 4 and increased it in clone 80 at the end of the OTC experiment.

CO_2 and O_3 effects on leaf litter C:N, C:P and lignin + polyphenolic:N

Elevated CO_2 treatments increased leaf litter C:N, but decreased C:P in both clones (Table 2). However, the CO_2-induced increase in C:N of clone 4 leaf litter diminished as the OTC experiment progressed, while in clone 80 the CO_2 effect was most obvious in leaf litter produced in 2001 (Figure 1 a). In contrast to CO_2-induced increase in C:N, the CO_2-caused decrease in C:P was most obvious in leaf litter collected from both clones in 1999, but thereafter this CO_2 effect either diminished or totally disappeared (Figure 1b). A clear CO_2-induced increase in lignin + polyphenolic:N (Table 2) was constant in clone 4 trees, but in clone 80 the positive CO_2 effect became more apparent at the end of the experiment (Figure 1c). In addition, in both clones this lignin + polyphenolic:N increase was similar in EC and in EC + EO trees which indicates that elevated O_3 did not diminish the positive effect of CO_2 in the combination treatment (Table 2).

C:P showed a significant overall O_3 effect as well, but this increase in C:P was seen in EO

Table 2. Nutrient concentrations, C:N, C:P and lignin + polyphenolic:N in leaf litter collected from chambers during the experiment period of 1999–2001 (CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO elevated CO_2 and O_3 in combination; $n = 4$ per treatment)

Variable	Clone		CC	EC	EO	EC + EO
N (mg g^{-1})	clone 4		9.31 ± 0.20	8.02 ± 0.17	8.33 ± 0.64	8.50 ± 0.24
	clone 80		8.24 ± 0.28	7.53 ± 0.31	8.21 ± 0.42	7.53 ± 0.33
P (mg g^{-1})	clone 4		3.53 ± 0.38	4.15 ± 0.13	2.98 ± 0.23	4.01 ± 0.22
	clone 80		4.31 ± 0.17	5.06 ± 0.26	3.70 ± 0.31	4.51 ± 0.49
K (mg g^{-1})	clone 4		8.40 ± 0.95	8.27 ± 0.66	7.98 ± 0.35	8.25 ± 0.52
	clone 80		6.77 ± 0.57	6.18 ± 0.84	7.62 ± 0.31	5.98 ± 0.45
Ca (mg g^{-1})	clone 4		8.94 ± 0.38	8.21 ± 0.54	8.10 ± 0.15	8.28 ± 0.40
	clone 80		13.07 ± 1.30	12.71 ± 0.76	11.02 ± 1.07	12.68 ± 1.47
Mg (mg g^{-1})	clone 4		3.09 ± 0.33	2.82 ± 0.21	2.91 ± 0.15	2.96 ± 0.18
	clone 80		4.31 ± 0.52	4.25 ± 0.30	3.69 ± 0.29	4.15 ± 0.32
Mn (mg g^{-1})	clone 4		1.34 ± 0.21	1.52 ± 0.10	1.03 ± 0.20	1.14 ± 0.21
	clone 80		1.43 ± 0.15	1.62 ± 0.09	1.33 ± 0.32	1.30 ± 0.20
Fe (μg g^{-1})	clone 4		245.0 ± 24.6	235.7 ± 18.4	269.7 ± 28.3	244.2 ± 20.2
	clone 80		201.3 ± 10.7	184.6 ± 24.5	176.3 ± 12.8	178.1 ± 11.2
Zn (μg g^{-1})	clone 4		458.8 ± 59.8	552.7 ± 84.8	415.6 ± 60.1	434.6 ± 15.0
	clone 80		563.2 ± 57.3	565.4 ± 74.9	450.8 ± 52.7	491.8 ± 31.0
Cu (μg g^{-1})	clone 4		6.03 ± 0.18	5.55 ± 0.22	5.51 ± 0.29	5.91 ± 0.52
	clone 80		5.63 ± 0.35	5.25 ± 0.33	5.37 ± 0.17	5.26 ± 0.26
B (μg g^{-1})	clone 4		40.08 ± 2.07	44.91 ± 1.69	38.85 ± 2.58	37.13 ± 2.26
	clone 80		55.52 ± 2.54	51.44 ± 5.97	51.74 ± 3.17	43.93 ± 3.23
S (μg g^{-1})	clone 4		715.7 ± 17.2	635.6 ± 12.2	644.2 ± 34.3	650.4 ± 23.9
	clone 80		637.6 ± 7.6	579.9 ± 8.1	638.8 ± 23.8	580.8 ± 24.1
C:N	clone 4		58.71 ± 0.99	67.20 ± 1.30	66.48 ± 4.93	63.48 ± 1.79
	clone 80		65.25 ± 2.05	71.53 ± 2.66	66.34 ± 3.49	71.42 ± 3.32
C:P	clone 4		164.3 ± 17.5	131.9 ± 5.7	189.9 ± 13.9	136.9 ± 7.3
	clone 80		128.5 ± 7.0	107.4 ± 4.9	149.8 ± 13.0	124.5 ± 15.8
Lignin:N	clone 4		49.98 ± 0.91	59.83 ± 0.85	56.65 ± 3.53	57.20 ± 1.78
	clone 80		56.35 ± 1.58	64.93 ± 3.07	58.22 ± 2.95	63.03 ± 1.72
Variable	Clone		CO_2	O_3	Clone×CO_2	CO_2×O_3
N	0.014	4 > 80	0.022[b]	[a]	[a]	[a]
P	0.002	4 < 80	0.001[c]	0.036[b]	[a]	[a]
K	< 0.0005	4 > 80	0.036[b]	[a]	0.019[f]	[a]
Ca	< 0.0005	4 < 80	[a]	[a]	[a]	[a]
Mg	< 0.0005	4 < 80	[a]	[a]	[a]	[a]
Mn	[a]		[a]	0.055[e]	[a]	[a]
Fe	< 0.0005	4 > 80	[a]	[a]	[a]	[a]
Zn	[a]		[a]	0.070[e]	[a]	[a]
Cu	[a]		[a]	[a]	[a]	[a]
B	< 0.0005	4 < 80	[a]	0.034[b]	[a]	[a]
S	0.002	4 > 80	0.004[b]	[a]	[a]	[a]
C:N	0.032	4 < 80	0.064[d]	[a]	[a]	[a]
C:P	0.007	4 > 80	< 0.0005[b]	0.046[c]	[a]	[a]
Lignin:N	0.002	4 < 80	0.001[c]	[a]	[a]	0.052[g]

Values are mean ± SE over a three-year period (units mg g^{-1} or μg g^{-1} of leaf d wt). *p*-values are from the between-subjects effects test in RM-ANOVA.

[a] $p > 0.1$, Significant [b]decrease, [c]increase, [d]marginally significant increase, [e]marginally significant decrease, [f]CO_2, but cl CO_2 response < c1 80 CO_2 response, [g]EC ≈ EC + EO and CO_2 effect > O_3 effect.

Table 3. Temporal clone, CO_2 and O_3 response patterns in N, K, Mn, B and S concentrations of leaf litter collected from chambers in 1999, 2000 and 2001 (CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO elevated CO_2 and O_3 in combination; $n = 4$ per treatment)

Treatment Clone	N mg g^{-1} 4	N mg g^{-1} 80	K mg g^{-1} 4	K mg g^{-1} 80	Mn mg g^{-1} 4	Mn mg g^{-1} 80	B μg g^{-1} 4	B μg g^{-1} 80	S μg g^{-1} 4	S μg g^{-1} 80
1999										
CC	10.63 ± 0.68	8.90 ± 0.43	6.11 ± 0.82	4.04 ± 0.23	1.63 ± 0.29	1.47 ± 0.20	27.05 ± 1.44	33.78 ± 3.31	794.0 ± 35.5	687.8 ± 12.4
EC	8.35 ± 0.41	8.60 ± 0.56	6.18 ± 0.75	3.26 ± 0.44	1.71 ± 0.05	1.91 ± 0.18	31.68 ± 2.69	34.20 ± 4.87	661.8 ± 18.4	654.8 ± 34.1
EO	9.40 ± 1.09	8.93 ± 0.48	5.91 ± 0.74	5.35 ± 0.20	1.20 ± 0.21	1.73 ± 0.43	27.43 ± 0.58	38.10 ± 1.59	720.5 ± 42.3	595.8 ± 22.8
EC + EO	8.83 ± 0.43	8.58 ± 0.34	5.85 ± 0.41	3.42 ± 0.12	1.36 ± 0.26	1.54 ± 0.26	26.00 ± 2.82	29.38 ± 1.94	695.3 ± 43.7	637.0 ± 31.2
2000										
CC	8.33 ± 0.13	7.48 ± 0.54	9.02 ± 1.08	7.32 ± 0.74	1.34 ± 0.22	1.42 ± 0.16	42.63 ± 3.23	60.65 ± 5.16	679.0 ± 19.5	579.8 ± 18.3
EC	7.33 ± 0.22	6.63 ± 0.31	8.65 ± 0.78	7.43 ± 1.14	1.41 ± 0.11	1.49 ± 0.07	43.20 ± 2.92	52.80 ± 6.91	671.0 ± 25.1	513.8 ± 5.4
EO	7.75 ± 0.66	8.05 ± 0.47	8.53 ± 0.45	8.50 ± 0.44	0.94 ± 0.23	1.11 ± 0.31	41.00 ± 2.32	53.53 ± 3.64	715.8 ± 18.2	608.0 ± 27.4
EC + EO	8.08 ± 0.39	7.05 ± 0.45	9.03 ± 0.59	6.76 ± 0.63	1.09 ± 0.19	1.13 ± 0.19	38.78 ± 1.74	43.90 ± 4.31	672.0 ± 19.4	529.0 ± 30.1
2001										
CC	8.98 ± 0.23	8.35 ± 0.19	10.06 ± 1.23	8.95 ± 0.76	1.06 ± 0.16	1.39 ± 0.11	50.55 ± 4.09	72.13 ± 2.36	665.3 ± 26.6	654.0 ± 17.2
EC	8.38 ± 0.23	7.35 ± 0.28	10.00 ± 0.80	7.84 ± 0.99	1.46 ± 0.15	1.48 ± 0.14	59.85 ± 4.14	67.33 ± 6.40	590.3 ± 8.2	555.0 ± 23.8
EO	7.83 ± 0.27	7.65 ± 0.36	9.52 ± 0.41	9.02 ± 0.36	0.94 ± 0.20	1.14 ± 0.24	48.13 ± 6.32	63.60 ± 5.30	616.3 ± 43.2	592.5 ± 30.0
EC + EO	8.60 ± 0.35	6.95 ± 0.25	9.89 ± 0.62	7.76 ± 0.86	0.97 ± 0.20	1.22 ± 0.17	46.60 ± 4.31	58.50 ± 4.48	619.0 ± 28.7	541.3 ± 25.9

Values are mean ± SE (units mg g^{-1} or μg g^{-1} of leaf d wt).

Table 4. Cell wall chemistry in leaf litter collected from chambers during the experiment period of 1999–2001 (CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO elevated CO_2 and O_3 in combination; $n = 4$ per treatment)

Variable	Clone		CC	EC	EO	EC + EO
Total sugars %	cl 4		19.66 ± 0.17	20.06 ± 0.29	20.32 ± 0.34	20.08 ± 0.35
	cl 80		19.38 ± 0.13	19.68 ± 0.26	19.47 ± 0.29	19.72 ± 0.59
α-cellulose %	cl 4		12.32 ± 0.09	11.92 ± 0.29	12.48 ± 0.29	11.67 ± 0.23
	cl 80		10.87 ± 0.10	10.80 ± 0.18	10.90 ± 0.17	10.53 ± 0.11
Hemicellulose %	cl 4		16.54 ± 0.20	16.95 ± 0.44	16.85 ± 0.46	17.33 ± 0.30
	cl 80		17.96 ± 0.16	18.42 ± 0.29	17.95 ± 0.27	18.89 ± 0.64
Uronic acids %	cl 4		9.19 ± 0.19	8.81 ± 0.11	9.01 ± 0.22	8.92 ± 0.18
	cl 80		9.45 ± 0.13	9.54 ± 0.17	9.38 ± 0.19	9.69 ± 0.10
Total lignin + polyphenolics %	cl 4		46.07 ± 0.53	47.65 ± 0.82	46.08 ± 0.51	48.28 ± 0.31
	cl 80		46.01 ± 0.87	48.06 ± 0.56	47.32 ± 0.73	46.93 ± 0.92
Gravimetric lignin %	cl 4		37.65 ± 0.38	39.08 ± 0.70	37.50 ± 0.48	39.55 ± 0.27
	cl 80		36.84 ± 0.65	38.57 ± 0.31	37.47 ± 0.70	38.23 ± 0.88
Acid-soluble lignin %	cl 4		8.42 ± 0.20	8.57 ± 0.23	8.58 ± 0.18	8.73 ± 0.24
	cl 80		9.18 ± 0.37	9.49 ± 0.27	9.85 ± 0.23	8.70 ± 0.23
Acetone-soluble extractives %	cl 4		12.57 ± 0.22	12.73 ± 0.32	12.76 ± 0.17	12.01 ± 0.48
	cl 80		12.55 ± 0.40	11.61 ± 0.93	12.58 ± 0.46	11.22 ± 0.66
Variable	Clone		CO_2	Clone×CO_2	CO_2×O_3	Clone×CO_2×O_3
Total sugars	0.056	4 > 80	[a]	[a]	[a]	[a]
α-cellulose	< 0.0005	4 > 80	0.007[b]	[a]	[a]	[a]
Hemicellulose	< 0.0005	4 < 80	0.041[c]	[a]	[a]	[a]
Uronic acids	< 0.0005	4 < 80	[a]	0.071[d]	[a]	[a]
Total lignin + polyphenolics	[a]		0.013[c]	[a]	[a]	[a]
Gravimetric lignin	[a]		0.001[c]	[a]	[a]	[a]
Acid-soluble lignin	< 0.0005	4 < 80	[a]	[a]	0.048[e]	0.051[f]
Acetone-soluble extractives	0.041	4 > 80	0.014[b]	0.038[d]	[a]	[a]

Values are mean ± SE over a three-year period (units % of leaf d wt). *p*-values are from the between-subjects effects test in RM-ANOVA

[a] $p > 0.1$, Significant [b]decrease, [c]increase, [d]cl 4 CO_2 response ≠ or < cl 80 CO_2 response, [e]EC↑ and EO↑ in cl 4 and cl 80, [f]but cl 4 EC + EO ≠ cl 80 EC + EO.

trees' leaf litter only (Table 2). In 2001, elevated O_3 increased C:N and lignin + polyphenolic:N in both clones (Figure 1a, c), although in C:N this increase was mainly due to clone 80 O_3 response.

CO_2 and O_3 effects on early decomposition dynamics in litter-bag Incubations 1–3

After approximately 11 months (i.e. at the final harvests of litter-bags), the total average leaf litter mass remaining in the bags was 52% in Incubation 1, 53% in Incubation 2 and 61% in Incubation 3. During Incubation 3, the total cumulative rainfall over the whole study period was lower (505 mm) than in Incubations 1 and 2 (cumulative rainfall 569 and 592 mm, respectively). In addition, the average air temperatures during August and September of year 2002 were higher (+17.1 and +14.7 °C) than those during August and September in year 2000 (+13.9 and +8.4 °C) and 2001 (+14.6 and +12.3 °C). The warmer and drier autumn might thus explain why the litter mass loss was slightly smaller at the end of the Incubation 3 than in the two previous litter-bag incubations.

In Incubation 1 (1999–2000), a significant overall clone×CO_2 interaction effect on leaf litter decomposition was observed, as clone 4 leaf litter produced under elevated CO_2 decomposed least and clone 80 leaves grown under EC- and EC + EO-treatments decomposed most (Figure 2a). In Incubation 2 (2000–2001), litter-bags filled with leaf litter produced under elevated CO_2 had higher remaining leaf litter mass than those filled with leaves grown in ambient CO_2. Although this adverse CO_2 effect was mainly observed in clone 4

Table 5. Temporal clone, CO_2 and O_3 response patterns in cell wall chemistry of leaf litter collected from chambers in 1999, 2000 and 2001 (CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO elevated CO_2 and O_3 in combination; $n = 4$ per treatment)

Treatment	α-cellulose %	α-cellulose %	Hemi-cellulose %	Hemi-cellulose %	Acid-soluble lignin %	Acid-soluble lignin %	Uronic acids %	Uronic acids %
Clone	4	80	4	80	4	80	4	80
1999								
CC	11.96 ± 0.38	10.24 ± 0.21	14.98 ± 0.27	17.24 ± 0.41	8.51 ± 0.25	9.74 ± 0.25	7.91 ± 0.28	9.33 ± 0.16
EC	12.33 ± 0.53	10.15 ± 0.42	15.55 ± 0.45	16.90 ± 0.35	8.36 ± 0.22	9.57 ± 0.29	8.19 ± 0.28	8.77 ± 0.48
EO	12.23 ± 0.64	10.69 ± 0.24	16.11 ± 0.36	17.03 ± 0.51	8.64 ± 0.19	10.56 ± 0.41	8.45 ± 0.36	9.25 ± 0.27
EC + EO	11.16 ± 0.37	10.33 ± 0.25	16.58 ± 0.28	17.99 ± 0.67	9.04 ± 0.44	9.41 ± 0.35	8.43 ± 0.11	9.42 ± 0.14
2000								
CC	13.10 ± 0.28	11.36 ± 0.30	17.13 ± 0.46	19.02 ± 0.17	8.44 ± 0.21	9.39 ± 0.31	9.78 ± 0.25	10.35 ± 0.27
EC	12.11 ± 0.35	11.57 ± 0.49	17.22 ± 0.48	20.10 ± 0.39	8.67 ± 0.16	9.16 ± 0.35	8.88 ± 0.12	10.65 ± 0.10
EO	12.92 ± 0.39	11.06 ± 0.29	17.69 ± 0.47	18.79 ± 0.13	8.86 ± 0.11	10.16 ± 0.32	9.09 ± 0.28	9.82 ± 0.06
EC + EO	12.67 ± 0.50	10.69 ± 0.45	17.47 ± 0.49	19.89 ± 1.20	8.31 ± 0.54	7.58 ± 0.41	9.29 ± 0.35	10.01 ± 0.20
2001								
CC	11.88 ± 0.31	11.03 ± 0.20	17.52 ± 0.05	17.62 ± 0.57	8.30 ± 0.26	8.40 ± 0.61	9.89 ± 0.17	8.67 ± 0.15
EC	11.30 ± 0.34	10.69 ± 0.19	18.08 ± 1.19	18.26 ± 0.71	8.68 ± 0.51	9.74 ± 0.40	9.35 ± 0.17	9.21 ± 0.29
EO	12.30 ± 0.24	10.95 ± 0.17	16.75 ± 0.67	18.02 ± 0.61	8.26 ± 0.46	8.83 ± 0.37	9.49 ± 0.39	9.06 ± 0.40
EC + EO	11.19 ± 0.25	10.56 ± 0.17	17.95 ± 0.55	18.78 ± 0.60	8.83 ± 0.16	9.12 ± 0.63	9.05 ± 0.34	9.66 ± 0.40

Values are mean ± SE over a three-year period (units % of leaf d wt).

EC and EC + EO leaf litter (Figure 2b), also clone 80 leaf litter grown under EC treatment had retarded decomposition, and therefore clonal difference in CO_2 response was not as apparent as it was in Incubation 1 (Figure 2a).

In Incubation 3 (2001–2002), clone 4 leaves grown under EO- and EC + EO-treatments had higher remaining leaf litter mass than those produced under ambient O_3 levels, whereas in clone 80 the reduction in the decomposition of leaf litter due to O_3 was only negligible (Figure 2c). However, at the final litter-bag harvest (9/9/2002), leaf litter produced under elevated O_3 had a tendency to decompose slower than that produced under ambient O_3 air (Figure 3a–b).

Clone effect on leaf litter quality and early decomposition dynamics

Leaf litter harvested from clone 4 trees had higher initial N, K, Fe and S concentrations, but lower P, Ca, Mg and B concentrations than leaf litter collected from clone 80 trees (Table 2). However, the clonal difference diminished in leaf litter K and Fe concentrations (Fe concentrations not shown) and increased in B concentrations as the OTC experiment progressed (year×clone effects, $p < 0.0005$, $p = 0.024$ and $p = 0.021$ for K, Fe and B concentrations, Table 3). Total sugar, α-cellulose and acetone-soluble extractive concentrations were higher, but hemicellulose, uronic acid and acid-soluble lignin concentrations lower in clone 4 than in clone 80 leaf litter (Table 4). Most of these clonal differences (e.g. clonal differences in total sugars, α-cellulose, hemicellulose, uronic acid and acid-soluble lignin concentrations) diminished or even disappeared as the OTC experiment progressed (year×clone effects, $p = 0.075$, $p = 0.094$, $p = 0.071$, $p < 0.0005$ and $p = 0.024$, respectively, Table 5). Overall C:N and lignin + polyphenolic:N were lower and C:P higher in clone 4 than in clone 80 leaf litter (Table 2). Nevertheless, in C:N the clonal difference was clearest in 2001 and in C:P in 1999 (Figure 1a–b). Clonal difference in leaf litter decomposition was not observed in Incubation 1 (Figure 2a), but it became significant in the litter-bag Incubations 2 and 3, as clone 4 leaves decomposed clearly less than those of clone 80 (Figure 2b–c).

Correlations between leaf litter quality and early decomposition dynamics

Table 6 shows how leaf litter quality parameters (nutrients and cell wall chemistry parameters) are loaded within different leaf litter quality

components (LQ1–LQ5) in the PCA analysis. The values in rotated pattern matrix are the correlation coefficients between the explanatory factor and the rotated component. Large loadings for particular factors within a component indicate that they can have a simultaneous effect. For instance, in 1999–2001 period, N, S, C:N and lignin + polyphenolic:N were always loaded within the same litter quality components with each other (within component LQ2 in 1999, and within component LQ1 in 2000 and 2001). Similarly, Mn, P and C:P (within component LQ4 in 1999, and LQ2 in 2000 and 2001), and Mg and Ca concentrations had constantly simultaneous effect (Mg and Ca were loaded within component LQ1 in 1999, LQ3 in 2000, and LQ2 in 2001) throughout the study period of 1999–2001.

Correlation analysis showed that in Incubation 1 the remaining leaf litter mass % correlated significantly with LQ2 (e.g. initial N and S concen-

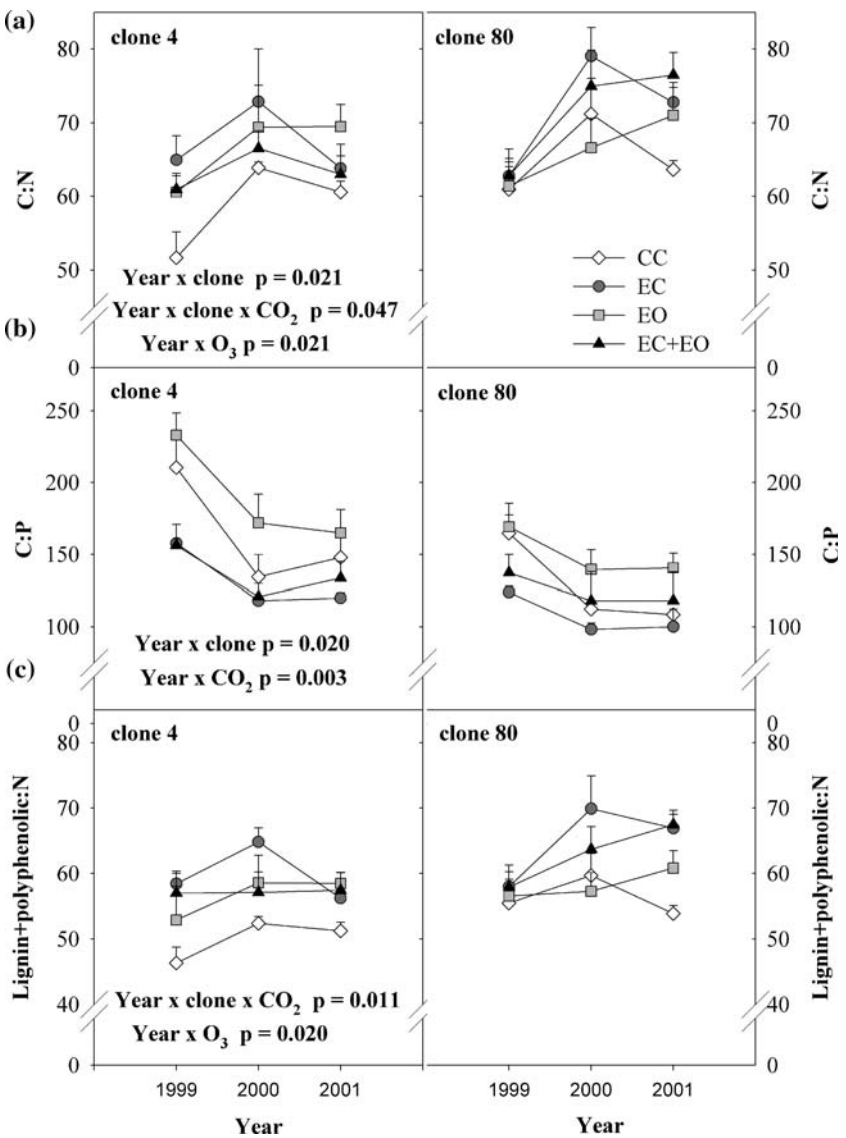

Figure 1. (a) Temporal response patterns in leaf litter C:N, (b) C:P and (c) lignin + polyphenolic:N in 1999, 2000 and 2001, respectively. Values are mean ± SE ($n = 4$ per treatment). Abbreviations: CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO = elevated CO_2 and O_3 in combination.

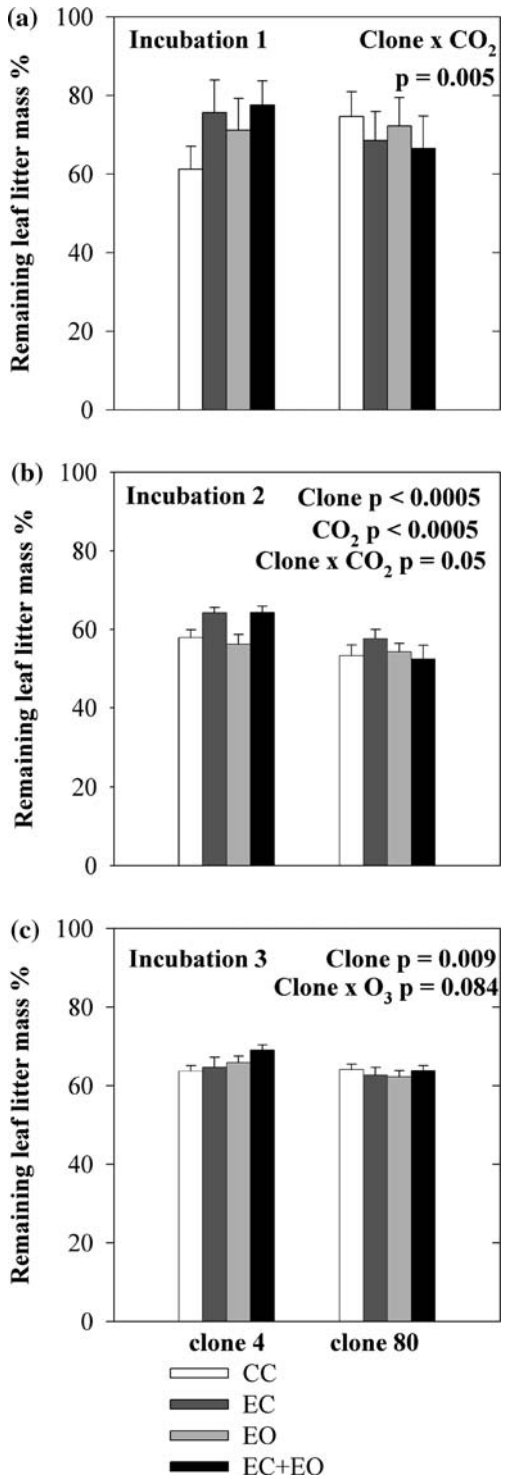

Figure 2. (a) Overall relative proportion of remaining leaf litter mass (%) in litter-bag incubation 1, (b) in litter-bag incubation 2 and (c) in litter-bag incubation 3. Values are mean ± SE ($n = 7$–20 per treatment). Abbreviations: CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO = elevated CO_2 and O_3 in combination.

trations and C:N and lignin + polyphenolic:N), this correlation being strongest after 255 field incubation days (Table 6). Furthermore, the opposite signs of the correlation coefficients within the LQ2 component indicate that the influence of N and S on decomposition was opposite to that of C:N and lignin + polyphenolic:N in general. In other words, leaf litter that had lower N and S concentrations, but simultaneously higher C:N and lignin + polyphenolic:N, decomposed less than leaf litter with higher N and S concentrations and lower C:N and lignin + polyphenolic:N.

In Incubation 2, there was a significant correlation between remaining leaf litter mass % and LQ3 (e.g. uronic acids, Ca, Mg, gravimetric lignin and B), this litter quality component effect

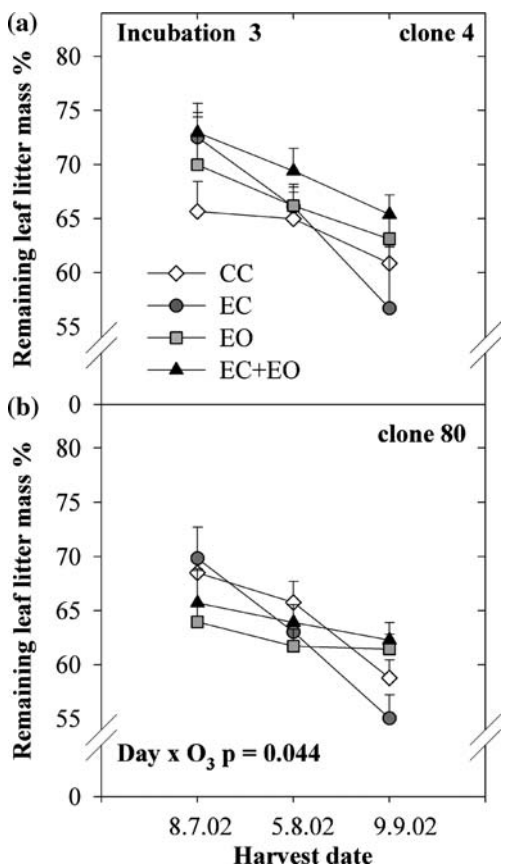

Figure 3. (a) Relative proportions of remaining leaf litter masses of clone 4 and (b) clone 80 after 263 (8.7.2002), 291 (5.8.2002) and 326 (9.9.2002) days in litter-bag incubation 3. Values are mean ± SE ($n = 3$–8 per treatment per harvest). Abbreviations: CC = chamber control, EC = elevated CO_2 alone, EO = elevated O_3 alone, EC + EO = elevated CO_2 and O_3 in combination.

Table 6. Rotated pattern matrix of Principal Component analysis (Varimax rotation) for leaf litter nutrients and cell wall chemistry in 1999–2001 as well as Pearson's correlation coefficients between leaf litter quality components (LQ1–LQ5) and remaining leaf litter mass (% of initial leaf litter mass) in Incubations 1–3*

Components 1999	LQ1	LQ2	LQ3	LQ4
K	−0.898			
α-cellulose	−0.888			
Acetone-soluble extractives	−0.711			
Total sugars	−0.679			
Ca	0.874			
Mg	0.854			
Hemicellulose	0.820			
Uronic acids	0.730			
N		−0.956		
S		−0.930		
C:N		0.978		
Lignin + polyphenolic:N		0.932		
Gravimetric lignin			−0.885	
Cu			−0.734	
Fe			−0.715	
B			0.794	
Acid-soluble lignin			0.677	
C:P				−0.585
Mn				0.954
Zn				0.665
P				0.588
Correlations				
Incubation 1 with 1999 leaf litter	LQ1	LQ2	LQ3	LQ4
Remaining mass % (after 255 days)	−0.322	**0.831**	0.143	−0.170
Remaining mass % (after 318 days)	−0.298	−0.160	0.013	−0.278
Remaining mass % (total average)	−0.252	**0.665**	0.136	−0.150

being strongest after 320 days and gravimetric lignin effect on decomposition differing from the effects of the other factors within the LQ3 component (Table 6). Simultaneous increase in gravimetric lignin concentration, and decrease in nutrient concentrations (Ca, Mg and B) and in uronic acid concentration thus decreased leaf litter decomposition in Incubation 2.

In Incubation 3, LQ4 component (initial gravimetric lignin concentration) correlated with the remaining leaf litter mass % after the first harvest (Table 6), as leaf litter containing higher amount of gravimetric lignin also decomposed less. In addition, LQ2 component (C:P, Mn, P, B, α-cellulose, Mg, Ca, and hemicellulose) correlated with the remaining leaf litter mass % after the third harvest (Table 6). Initial C:P and α-cellulose effects on leaf litter decomposition were parallel (increase of both decreased decomposition), but their effects differed from those of the other factors (higher hemicellulose concentration and Mn, P, B, Mg and Ca concentrations enhanced decomposition).

Discussion

O_3 effects on leaf litter quality and early decomposition dynamics

Altogether, the present results suggest that overall O_3 effects seen in green foliage nutrient composition are reflected in leaf litter quality as well, but the magnitude of O_3 effect on nutrients was greater in green foliage than in leaf litter. O_3-caused decreases in P (in EO treatment only), Mn, Zn and B concentrations and increase in C:P (in EO treatment only) were seen before (Table 1) and after leaf abscission (Table 2). Since the total leaf mass of the experimental trees was

Table 6. Continued

Components 2000	LQ1	LQ2	LQ3	LQ4	LQ5
S	−0.928				
Cu	−0.927				
N	−0.919				
K	−0.737				
C:N	0.928				
Lignin + polyphenolic:N	0.909				
Hemicellulose	0.722				
C:P		−0.801			
Fe		−0.718			
Mn		0.944			
Zn		0.884			
P		0.752			
Gravimetric lignin			−0.806		
Uronic acids			0.888		
Ca			0.832		
Mg			0.824		
B			0.642		
Acid-soluble lignin				0.851	
Acetone-soluble extractives				0.639	
Total sugars					0.809
α-cellulose					0.691
Correlations					
Incubation 2 with 2000 leaf litter	LQ1	LQ2	LQ3	LQ4	LQ5
Remaining mass % (after 259 days)	0.095	0.290	**−0.775**	−0.249	0.461
Remaining mass % (after 285 days)	−0.201	0.559	−0.475	0.038	0.305
Remaining mass % (after 320 days)	−0.356	0.174	**−0.828**	−0.324	−0.030
Remaining mass % (total average)	−0.237	0.416	**−0.774**	−0.204	0.177

not significantly affected by the O_3 exposure in the OTC experiment (Kasurinen et al., unpublished data, Riikonen et al., 2004), it is reasonable to assume that O_3-induced reductions in P, Mn, Zn and B concentrations could lead to decreased contents and thereby decreased inputs of these nutrients into the soil. In fact, leaf litter B and Mn contents decreased, while leaf litter C:P increased under elevated O_3, all of these trends being mainly apparent in EO trees (Kasurinen et al., unpublished data).

In contrast to nutrients, cell wall chemistry changes due to O_3 stress were minor. O_3 increased acid-soluble lignin concentration in leaf litter of both clones constantly almost throughout the whole experiment, but only under ambient CO_2 (i.e. in EO treatment only), while in green foliage similar response due to O_3 (either EO alone or in combination with CO_2) was not observed (Oksanen et al., 2005).

Some of the O_3 effects on leaf litter quality of both clones became stronger (e.g. decreases in B and N concentrations) or visible (e.g. increases in C:N and lignin + polyphenolics:N) only after the last experiment season. In addition, O_3-caused decrease in Mn concentrations became apparent in both clones in 2001. Partly parallel with above-mentioned litter quality data, a negative effect of O_3 on leaf litter mass loss in the field incubations was found in Incubation 3 only. Thus, at the last litter-bag harvest (after 326 field incubation days) O_3 decreased decomposition of leaf litter in both clones (Figure 3). Correlation analysis (Table 6) shows that LQ2 component (component included Mn, P, B and C:P, all of which were more or less affected by elevated O_3 in 2001), correlated negatively with the leaf litter decomposition at this third and final harvest. This result indicates that O_3-induced changes in the initial chemical composition of leaf litter

Table 6. Continued

Components 2001	LQ1	LQ2	LQ3	LQ4	LQ5
C:N	−0.963				
Lignin + polyphenolic:N	−0.918				
Acid-soluble lignin	−0.614				
S	0.970				
N	0.959				
Cu	0.870				
K	0.759				
C:P		−0.940			
α-cellulose		−0.681			
Mn		0.937			
P		0.919			
B		0.790			
Mg		0.677			
Ca		0.652			
Hemicellulose		0.593			
Acetone-soluble extractives			0.838		
Fe			0.797		
Zn			0.700		
Gravimetric lignin				0.942	
Uronic acids					−0.889
Total sugars					0.869
Correlations					
Incubation 3 with 2001 leaf litter	LQ1	LQ2	LQ3	LQ4	LQ5
Remaining mass % (after 263 days)	0.178	0.043	0.483	**0.659**	0.341
Remaining mass % (after 291 days)	0.523	−0.317	0.121	0.409	0.291
Remaining mass % (after 326 days)	0.078	**−0.831**	−0.457	−0.005	0.159
Remaining mass % (total average)	0.315	−0.492	−0.012	0.487	0.350

*In rotated pattern matrix only high loadings (i.e. values below −0.550 or above +0.550) to each component are shown. Bolded values in correlation matrix indicate a significant or marginally significant correlation between the leaf litter mass loss and leaf litter quality component ($n = 8$).

might have been at least partially responsible for the decreased mass loss during the field incubation. Our result is in contradiction with the results of other field experiments in which either the chemical composition or subsequent decomposition of leaf litter produced under elevated O_3 was not found to be altered significantly (Kainulainen et al., 2003; Parsons et al., 2004).

Leaf litter quality after CO_2 exposure

As with elevated O_3, the magnitude of CO_2 effect was usually larger in green foliage than in leaf litter. Nevertheless, the majority of the CO_2-induced changes in green leaf nutrient concentrations were manifested in the leaf litter quality as well (Tables 1 and 2). For instance, CO_2-induced reductions in overall leaf N and S were parallel to those observed in green leaf samples taken from the same experimental trees just before the maximum leaf abscission period (Table 1) and earlier in the growing season (Oksanen et al., 2005, Riikonen et al., 2005). In addition, the CO_2-induced increases in P concentrations were observed in leaves collected before and after leaf abscission (Tables 1 and 2). Although K concentrations were decreased due to CO_2 in both green leaves (Table 1, Oksanen et al., 2005) and in leaf litter, there was also a clear clonal difference in CO_2 response of K concentrations in leaf litter (e.g. CO_2-induced decrease in K concentrations of leaf litter was mainly due to clone 80 negative

CO_2 response). Other studies have also reported that CO_2-induced decrease in leaf N concentrations persists through the leaf senescence (De Angelis et al., 2000, Johnson et al., 2001) and therefore decreases in leaf litter N concentrations due to CO_2 could be expected (Norby et al., 2001). However, in contrast to our study results, significant CO_2-induced decreases or increases of other nutrients have not been usually observed (Cotrufo et al., 1998a; Coûteaux et al., 1995).

In general, CO_2-induced reductions in nutrient concentrations in green foliage and leaf litter are usually explained by the dilution effect as leaf dry mass changes due to accumulation of non-structural carbohydrates (Gifford et al., 2000; Norby et al., 2000, 2001). However, although CO_2-caused starch accumulation and increase of leaf dry weight were observed in the present study as well (Riikonen et al., 2005), it was suggested that the initial CO_2-induced N decrease in foliage was more linked to reduced amount and activity of Rubisco as well as down-regulation of photosynthesis (Eichelmann et al., 2004, Oksanen et al., 2005, Riikonen et al., 2005). Of other nutrients, CO_2-caused decreases in K and S could be related to the observed down-regulation of photosynthesis as well, as both of these nutrients are essential for the functioning of the photosynthetic machinery (Marschner, 1995, Oksanen et al., 2005). Finally, stomatal conductance and thereby also transpiration flow was reduced under elevated CO_2 (Riikonen et al., 2005), which might have led to less efficient uptake of these nutrients. On the other hand, elevated CO_2 increased leaf litter production in clone 80 trees (Kasurinen et al., unpublished data, Riikonen et al., 2004). Based on this information, it could be assumed that at least in clone 80 trees elevated CO_2 could compensate for these decreases in nutrient concentrations by simultaneously increasing leaf litter inputs into the soil. Although nutrient contents in general did not show statistically significant clonal differences in their CO_2 responses, CO_2 increased nutrient contents more consistently in clone 80 leaf litter than in clone 4 leaf litter (Kasurinen et al., unpublished data). Thus, in clone 80 trees CO_2-induced increase in leaf litter production could compensate for the decreased N, S and K concentrations under elevated CO_2, whereas in clone 4 the CO_2-induced litter mass increment and compensation effects on N and S remained relatively small.

In contrast to N, plant P requirement for photosynthetic machinery is not necessarily down-regulated under elevated CO_2 (Conroy and Hocking, 1993). In other words, if elevated CO_2 increases photosynthesis, as it did in the present study (Riikonen et al., 2005), there can be an increased P requirement for the phosphorylated photosynthetic intermediates and intercellular transport (Gifford et al., 2000). A recent FACE experiment conducted with loblolly pine and four deciduous tree species (red bud, dogwood, red maple and sweet gum) supports partially our finding that leaf litter P concentrations can in fact increase under CO_2, but in contrast to our study, this two-year experiment also indicated that the effects of elevated CO_2 on green foliage P (no CO_2-induced increase) and leaf litter P differed, and thus suggested that the resorption efficiency of P was decreased under elevated CO_2 (Finzi et al., 2001).

CO_2-induced increase of C:N was already seen in green foliage (Riikonen et al., 2005, Table 1), and C:P was also decreased both before and after leaf abscission under CO_2 treatments (Tables 1 and 2). CO_2-induced changes in C:P have been studied less than those in C:N, and usually the results have been contradictory. For instance, in some cases changes to green leaf C:P have not been necessarily transmitted into leaf litter quality, or sometimes pronounced CO_2 effects have been seen in leaf litter only, CO_2 then usually decreasing leaf litter C:P (Gifford et al., 2000). Nevertheless, also content values showed that leaf litter C:N and C:P status changed. Thus, although elevated CO_2 increased nutrient contents, it also simultaneously increased the total carbon content in leaf litter, and in that way increased the C:N and decreased the C:P status in CO_2-exposed leaf litter (Kasurinen et al., unpublished data).

Elevated CO_2 also affected cell wall chemistry significantly by decreasing α-cellulose concentration and increasing hemicellulose and lignin + polyphenolic concentrations simultaneously (Table 4). Oksanen et al. (2005) also reported a decrease in α-cellulose concentration and an increase in hemicellulose concentration of green leaves during exposure season 2001, but in contrast to our results, they observed an inconsistent and temporally varying CO_2 effect on total lignin + polyphenolic concentrations only (Oksanen et al., 2005). In the present experiment, C:N and

lignin + polyphenolic:N changes under elevated CO_2 were parallel with the CO_2-induced increases in leaf litter C:N and lignin:N observed in potted birch seedlings exposed to elevated CO_2 in solardomes (Cotrufo and Ineson, 1996; Cotrufo et al., 1994), and observed during a FACE experiment with field-growing paper birch (Parsons et al., 2004). Nevertheless, the magnitude of the CO_2 effect on C:N and lignin + polyphenolic:N was clearly lower in our OTC experiment (the average increase was 10% in C:N and 15% in lignin + polyphenolic:N) than in the previous pot seedling studies (the average increase was 46% in C:N and 53% in lignin:N; O'Neill and Norby, 1996) or than in the FACE experiment (the average increase was 42% in C:N and 45% in lignin:N; Parsons et al., 2004).

Some chemical parameters of leaf litter also showed a clonal difference in their CO_2 response during the study period. For instance, CO_2 decreased the uronic acid concentrations in leaf litter collected from clone 4, while in clone 80 leaf litter CO_2 had a tendency to increase uronic acids concentrations, this trend becoming clearer in 2001. In addition, K concentrations and acetone-soluble extractive concentrations decreased more due to CO_2 in leaf litter of clone 80 than that of clone 4. Finally, the elevated CO_2 effects on N and S concentrations and C:N were most apparent in clone 4 after the first exposure season, whereas in clone 80 the effects of CO_2 on these parameters became more apparent in 2001. In green foliage the clonal differences in CO_2 response of chemical quality were not usually observed (Oksanen et al., 2005, Table 1). However, the previous data supports our finding that clone 80 was in general more affected by elevated CO_2 than clone 4 at the end of this OTC experiment, as the total tree growth, total leaf production, total leaf area, gas exchange and water-use efficiency were all higher in clone 80 CO_2 trees, whereas the effects of elevated CO_2 were either not observed in clone 4 or the CO_2 impacts were less obvious in clone 4 than in clone 80 (Riikonen et al., 2004, 2005). In addition, some discrepancies between green foliage and litter data can also be explained by the fact that the litter contained leaves from both long- and short-shoots, whereas Oksanen et al. (2005) reported the nutrients and cell wall chemistry of short-shoot leaves only. Although it is well known that the magnitude of the CO_2 effects on leaf litter chemical quality can vary among different tree species (Boerner and Rebbeck, 1995; Cotrufo et al., 1994, 1998a; Coûteaux et al., 1999, Norby et al., 2001), this study indicates that some intra-species variation in CO_2 response of leaf litter nutrients and cell wall chemistry is likely to occur as well.

Early decomposition dynamics are transiently affected by elevated CO_2?

Genotype dependent CO_2 effects on leaf litter mass loss were seen in Incubation 1, as CO_2 decreased the subsequent mass loss of clone 4 leaf litter, whereas the mass loss of clone 80 leaf litter was enhanced. This result is parallel with the leaf litter quality change, as leaf litter quality was changed more under CO_2 in clone 4 than in clone 80 during the 1999 season. In other words, simultaneous CO_2-induced decreases in N and S concentrations and increases in C:N and lignin + polyphenolic:N might explain the decreased mass loss of clone 4 EC and EC + EO leaf litter in Incubation 1 (Table 6, Figure 2a). In Incubation 2, elevated CO_2 retarded the subsequent decomposition in both clones at least to some extent (Figure 2b). Nevertheless, the mass loss of clone 4 EC and EC + EO leaf litter was still smaller than that of clone 80. Since only uronic acid concentration showed clonal difference in CO_2 response in 2000, it is possible that this leaf litter quality parameter explains a part of the observed clonal difference in decomposition response to CO_2. Thus, CO_2 decreased uronic acid concentrations in leaf litter of clone 4 only, and, based on the correlation analysis, one factor that could diminish leaf litter decomposition was the decreased amount of uronic acids (Table 6). In contrast to the above-mentioned results, other authors have reported non-significant changes in the subsequent decomposition of leaf litter produced under CO_2 and incubated in the field (Finzi et al., 2001; Hirschel et al., 1997; Kainulainen et al., 2003). On the other hand, recently Parsons et al. (2004) reported that the leaf litter mass loss of field-grown and CO_2-exposed paper birches was significantly lower than that of those birches grown under ambient CO_2, and that this negative CO_2 effect was even enhanced, when the litter was incubated in the elevated CO_2 plots.

In some litter quality parameters (e.g N, S, C:N and lignin + polyphenolic:N), the CO_2 impact on clone 80 increased and on clone 4 decreased towards the end of the OTC experiment. However, in leaf litter decomposition (Incubation 3) this clonal difference in CO_2 response was not observed (Figure 2c). Since the correlation analysis showed that the above-mentioned chemical parameters (N, S, C:N and lignin + polyphenolic:N) correlated weakly with the mass loss in Incubation 3 (Table 6), the present decomposition result seems logical.

Clonal differences

In general, the majority of clonal differences in leaf chemical components persisted throughout the leaf senescence and abscission. For instance, clone 4 had lower leaf P, Ca, Mg and B concentrations, but higher K and S concentrations than clone 80, whereas clone 80 leaves had higher hemicellulose and uronic acid concentrations and lower α-cellulose concentration than clone 4 leaves before and after leaf abscission (Tables 1, 2 and 4; Oksanen et al., 2005). Nevertheless, in contrast to green foliage (Table 1; Oksanen et al., 2005), clone 4 leaf litter had higher N and Fe concentrations than that of clone 80, this difference between green foliage and leaf litter indicating that clone 4 was probably less efficient in N and Fe resorption than clone 80. This is not surprising, since other parameters (gas exchange and Rubisco activity) also showed that clone 80 was physiologically more active than clone 4 (Riikonen et al., 2003, 2005). Clonal difference in leaf cell wall chemistry is in agreement with previous studies made with wood material (Kaakinen et al., 2004; Pot et al. 2002), where cell wall chemistry was found to be genotype-dependent.

Due to lower N and higher P concentrations, clone 4 leaf litter also had higher C:N and lignin + polyphenolic:N and lower C:P than clone 80. Clone 4 leaves also decomposed slower than those of clone 80, but this clonal difference in decomposition was significant in Incubations 2 and 3 only (Figure 2). Correlation analysis (Table 6) indicated that Ca, Mg and B concentrations had also a simultaneous effect on litter decomposition (lower concentrations of Ca, Mg and B decreased mass loss), and since these nutrients also showed a clear clonal difference (Table 2), they seem to be the best explanatory factors for the observed clonal differences in decomposition in Incubations 2 and 3.

Conclusions

Elevated CO_2 altered the chemical composition of leaf litter in both clones more than elevated O_3. Based on concentration values, CO_2 effects on clone 80 leaf litter quality became gradually more apparent, whereas CO_2 effects on clone 4 leaf litter weakened as the OTC experiment progressed. In addition to CO_2 effect on leaf litter quality, also the retarding effect of CO_2 on early decomposition dynamics attenuated in clone 4 with time, whereas in clone 80 CO_2 effects on decomposition were inconsistent. Usually, whenever there was a clear CO_2 effect on leaf litter quality or decomposition, it was similar regardless of prevailing O_3 levels (i.e. elevated CO_2 alone and in combination with elevated O_3 caused similar responses). Furthermore, although elevated CO_2 simultaneously increased leaf litter production and thereby nutrient contents to some extent in both clones, also C content of leaf litter increased, and thereby led to increased C:N and decreased C:P status in leaf litter produced under elevated CO_2. The present study also indicates that a three-year exposure to elevated O_3 could have some negative effects on leaf litter quality (decreased nutrient concentrations and contents and increased C:P) and leaf litter decomposition. However, it is not certain whether these O_3 stress effects persist over longer periods of time and are seen in the later stages of decomposition. In conclusion, it can be stated that elevated CO_2 and O_3 levels have the potential to affect leaf litter quality in northern birch forests, but the effects of CO_2 and O_3 on the total decomposition process remain to be elucidated. In addition, the magnitude of CO_2-induced changes in litter quality and decomposition may depend largely on the genotype composition of the silver birch populations.

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References

Andersen C P 2001 Understanding the role of ozone stress in altering below-ground processes. *In* Tree Physiology, Volume 2. Trends in European Forest Tree Physiology Research. Eds. S Huttunen, H Heikkilä, J Bucher, B Sundberg P Jarvis and R Matyssek. pp. 65–79. Kluwer Academic Publishers, Dorcdrecht.

Andersen C P 2003 Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol. 157, 213–228.

Anttonen S, Manninen A-M, Saranpää P, Kainulainen P, Linder S and Vapaavuori E 2002 Effects of long-term nutrient optimisation on stem wood chemistry in *Picea abies*. Trees-Struct. Funct. 16, 386–394.

Blaschke L, Forstreuter M, Sheppard L J, Leith I K, Murray M B and Polle A 2002 Lignification in beech (*Fagus sylvatica*) grown at elevated CO_2 concentrations: Interaction with nutrient availability and leaf maturation. Tree Physiol. 22, 469–477.

Boerner R E J and Rebbeck J 1995 Decomposition and nitrogen release from leaves if three hardwood species grown under elevated O_3 and/or CO_2. Plant Soil 170, 169–177.

Cabané M, Pireaux J-C, Léger E, Weber E, Dizengremel P, Pollet B and Lapierre C 2004 Condensed lignins are synthesized in poplar leaves exposed to ozone. Plant Physiol. 134, 586–594.

Cadisch G and Giller K E 1996 Driven by Nature. Plant Litter Quality and Decomposition. CAB International, Wallingford.

Ceulemans R and Mousseau M 1994 Effects of elevated atmospheric CO_2 on woody plants. New Phytol. 127, 425–446.

Conroy J and Hocking P 1993 Nitrogen nutrition of C3 plants at elevated CO_2 concentrations. Physiol. Plantarum 89, 570–576.

Cooley D R and Manning W J 1987 The impact of ozone assimilate partitioning in plants: A review. Environ. Pollut. 47, 95–113.

Cotrufo M F, Berg B and Kratz W 1998a Increased atmospheric CO_2 and litter quality. Environ. Rev. 6, 1–12.

Cotrufo M F, Briones M J I and Ineson P 1998b Elevated CO_2 affects field decomposition rate and palatability of tree leaf litter: Importance of changes in substrate quality. Soil Biol. Biochem. 30(12), 1565–1571.

Cotrufo M F and Ineson P 1996 Elevated CO_2 reduces field decomposition rates of *Betula pendula* (Roth.) leaf litter. Oecologia 106, 525–530.

Cotrufo M F, Ineson P and Rowland A P 1994 Decomposition of tree leaf litters grown under elevated CO_2: Effect of litter quality. Plant Soil 163, 121–130.

Cotrufo M F, Ineson P and Scott A 1998c Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biol. 4, 43–54.

Coûteaux M-M, Bottner P and Berg B 1995 Litter decomposition, climate and litter quality. Trends Ecol. Evol. 10, 63–66.

Coûteaux M-M, Kurz C, Bottner P and Raschi A 1999 Influence of increased atmospheric CO_2 concentration on quality of plant material and litter decomposition. Tree Physiol. 19, 301–311.

Coûteaux M-M, Monrozier L J and Bottner P 1996 Increased atmospheric CO_2: chemical changes in decomposing sweet chestnut (*Castanea sativa*) leaf litter incubated in microcosms under increasing food web complexity. Oikos 76, 553 563.

De Angelis P, Chigwerewe K S and Mugnozza G E S 2000 Litter quality and decomposition in a CO_2-enriched Mediterranean forest ecosystem. Plant Soil 224, 31–41.

Eichelmann H, Oja V and Rasulov B 2004 Photosynthetic parameters of birch (*Betula pendula* Roth) leaves growing in normal and in CO_2- and O_3-enriched atmospheres. Plant Cell Environ. 27, 479–495.

Findlay S, Carreiro M, Krischik V and Jones C G 1996 Effects of damage to living plants on leaf litter quality. Ecol. Appl. 6(1), 269–275.

Findlay S and Jones C G 1990 Exposure of cottonwood plants to ozone alters subsequent litter decomposition. Oecologia 82, 248–250.

Finzi A C, Allen A S, DeLucia E H, Ellsworth D S and Schlesinger W H 2001 Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. Ecology 82(2), 470–484.

Gifford R M, Barrett D J and Lutze J L 2000 The effects of elevated [CO_2] on the C:N and C:P mass ratios of plant tissues. Plant and Soil 224, 1–14.

Halonen O, Tulkki H and Derome J, 1983 Nutrient analysis methods. Metsäntutkimuslaitoksen tiedonantoja 121.

Hirschel G, Körner C and Arnone J A 1997 Will rising atmospheric CO_2 affect leaf litter quality and in situ decomposition rates in native plant communities? Oecologia 110, 387–392.

IPCC (Intergovernmental Panel on Climate Change) 2001 Climate change 2001: The scientific basis. Technical summary. 63 p. URL:http://www.ipcc.ch/pub/wg1TARtechsum.pdf.

Johnson D W, Norby R J and Hungate B A 2001 Effects of elevated CO_2 on nutrient cycling in forests. *In* The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems. Report No. 3 of the IUFRO Task Force on Environmental Change. Eds. D F Karnosky, R Ceulemans, G E S Mugnozza and J L Innes. pp. 237–267. CABI Publishing, CAB International, Wallingford.

Kaakinen S, Kostiainen K, Ek F, Saranpää P, Kubiske M E, Sobers J, Karnosky D F and Vapaavuori E 2004 Stem wood properties of *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* saplings after 3 years of treatments to elevated carbon dioxide and ozone. Global Change Biol. 10, 1513–1525.

Kainulainen P, Holopainen T and Holopainen J K 2003 Decomposition of secondary compounds from needle litter of Scots pine grown under elevated CO_2 and O_3. Global Change Biol. 9, 295–304.

Karnosky D F, Zak D R, Pregitzer K S, Awmack C S, Bockheim J G, Dickson R E, Hendrey G R, Host G E, King

J S, Kopper B J, Kruger E L, Kubiske M E, Lindroth R L, Mattson W J, McDonald E P, Noormets A, Oksanen E, Parsons W F J, Percy K E, Podila G K, Riemenschneider D E, Sharma P, Thakur R, Sober A, Sober J, Jones W S, Anttonen S, Vapaavuori E, Mankovska B, Heilman W and Isebrands J G 2003 Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: A synthesis of molecular to ecosystem results from the Aspen FACE project. Funct. Ecol. 17, 289–304.

Kasurinen A, Kokko-Gonzales P, Riikonen J, Vapaavuori E and Holopainen T 2004 Soil CO_2 efflux of two silver birch clones exposed to elevated CO_2 and O_3 levels during three growing seasons. Global Change Biol. 10, 1654–1665.

King J S, Pregitzer K S, Zak D R, Kubiske M E, Ashby J A and Holmes W E 2001 Chemistry and decomposition of litter from *Populus tremuloides* Michaux grown at elevated atmospheric CO_2 and varying N availability. Global Change Biol. 7, 65–74.

Kuokkanen K, Julkunen-Tiitto R, Keinänen M, Niemelä P and Tahvanainen P 2001 The effect of elevated CO_2 and temperature on the secondary chemistry of *Betula pendula* seedlings. Trees – Struct. Funct. 15, 378–384.

Landolt W, Günthardt-Goerg M S, Pfenninger I, Einig W, Hampp R, Maurer S and Matyssek R 1997 Effect of fertilization on ozone-induced changes in the metabolism of birch (*Betula pendula*) leaves. New Phytol. 137, 389–397.

Luoranen J, 2000 Control of growth and frost hardening of silver birch container seedlings. growth retardants, short day treatment and summer planting. The Finnish Forest Research Institute, Research Papers 777.

Marschner H 1995 Mineral Nutrition of Higher Plants. Academic Press, London.

Mutikainen P, Walls M, Ovaska J, Keinänen M, Julkunen-Tiitto R and Vapaavuori E 2000 Herbivore resistence in *Betula pendula*: Effect of fertilisation, defoliation, and plant genotype. Ecology 81, 49–65.

Norby R J and Cotrufo M F 1998 A question of litter quality. Nature 396(5), 17–18.

Norby R J, Cotrufo M F, Ineson P, O'Neill E G and Canadell J 2001 Elevated CO_2, litter chemistry, and decomposition: a synthesis. Oecologia 127, 153–165.

Norby R J, Long T M, Hartz-Rubin J S and O'Neill E G 2000 Nitrogen resorption in senescing tree leaves in a warmer, CO_2-enriched atmosphere. Plant and Soil 224, 15–29.

Norby R J, Wullchleger S D, Gunderson C A, Johnson D W and Ceulemans R 1999 Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ. 22, 683–714.

Oksanen E, Riikonen J, Kaakinen S, Holopainen T and Vapaavuori E 2005 Structural characteristics and chemical composition of birch *(Betula pendula*) leaves are modified by increasing CO_2 and ozone. Global Change Biol. 11, 732–748.

O'Neill E G and Norby R J 1996 Litter quality and decomposition rates of foliar litter produced under CO_2 enrichment. *In* Carbon Dioxide and Terrestrial Ecosystems. Eds. G W Koch and H A Mooney. pp. 87–103. Academic Press, San Diego.

Pääkkönen E, Holopainen T and Kärenlampi L 1997 Variation in ozone sensitivity among clones of *Betula pendula* and *Betula pubescens*. Environ. Pollut. 95, 37–44.

Parsons W F J, Lindroth R L and Bockheim J G 2004 Decomposition of *Betula papyrifera* leaf litter under the independent and interactive effects of elevated CO_2 and O_3. Global Change Biol. 10, 1666–1677.

Poorter H, Van Berkel Y, Baxter R, Den Hertog J, Dijkstra P, Gifford R M, Griffin K L, Roumet C, Roy J and Wong S C 1997 The effect of elevated CO_2 on chemical composition and construction costs of leaves of 27 C_3 species. Plant Cell Environ. 20, 472–482.

Pot D, Chantre G, Rozenberg P, Rodrigues J C, Jones Gwynn L, Pereira H, Hannrup B, Cahalan C and Plomion C 2002 Genetic control of pulp and timber properties in maritime pine (*Pinus pinaster* Ait). Ann. Forest Sci. 59, 563–575.

Pye J M 1988 Impacts of ozone on the growth and yield of trees: A review. J. Environ. Qual. 17(3), 347–360.

Riikonen J, Holopainen T, Oksanen E and Vapaavuori E 2005 Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO_2 and O_3 in the field. Tree Physiol. 25, 621–632.

Riikonen J, Lindsberg M-M, Holopainen T, Oksanen E, Lappi J, Peltonen P and Vapaavuori E 2004 Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. Tree Physiol. 24, 1227–1237.

Riikonen J, Oksanen E, Peltonen P, Holopainen T and Vapaavuori E 2003 Seasonal variation in physiological characteristics of two silver birch clones in the field. Can. J. Forest Res. 33, 2164–2176.

Saleem A, Loponen J, Pihlaja K and Oksanen E 2001 Effects of long-term open-field ozone exposure on leaf phenolics of European silver birch (*Betula pendula* Roth). J. Chem. Ecol. 27(5), 1049–1062.

Saxe H, Ellsworth D S and Heath J 1998 Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol. 139, 395–436.

SCAN-AM, 1994 Asetoniin liukenevan aineen määrittäminen (in Finnish). Scandinavian pulp. paper and board testing committee 50. Central Laboratory of Finland, Espoo, Finland.

Scherzer A J, Rebbeck J and Boerner R E J 1998 Foliar nitrogen dynamics and decomposition of yellow-poplar and eastern white pine during four seasons of exposure to elevated ozone and carbon dioxide. For. Ecol. Manag. 109, 355–366.

Swift M J, Heal O W and Anderson J M 1979 Decomposition in Terrestrial Ecosystems. Blackwell Scientific Publications, Oxford.

Vapaavuori E, Oksanen T, Holopainen J K, Holopainen T, Heiskanen J, Julkunen-Tiitto R, Kasurinen A, Oksanen E, Peltonen P, Poteri M, Repo T, Riikonen J and Syrjälä L, 2002 Technical report. Open-top chamber fumigation of cloned silver birch (Betula pendula Roth) trees to elevated CO2 and ozone: Description of the fumigation system and the experimental site. The Finnish Forest Research Institute, Research Papers 838. http://www.metla.fi/julkaisut/mt/838.

Yamaji K, Julkunen-Tiitto R, Rousi M, Freiwald V and Oksanen E 2003 Ozone exposure over two growing seasons alters root-to-shoot ratio and chemical composition of birch (*Betula pendula* Roth). Global Change Biol. 9, 1363–1377.

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