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Litter- and ecosystem-driven decomposition under elevated CO_2 and enhanced N deposition in a *Sphagnum* peatland

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A B S T R A C T

Peatlands represent massive global C pools and sinks. Carbon accumulation depends on the ratio between net primary production and decomposition, both of which can change under projected increases of atmospheric CO_2 and N deposition. The decomposition of litter is influenced by 1) the quality of the litter, and 2) the microenvironmental conditions in which the litter decomposes. This study aims at experimentally testing the effects of these two drivers in the context of global change. We studied the *in situ* litter decomposition from three common peatland species (*Eriophorum vaginatum*, *Polytrichum strictum* and *Sphagnum fallax*) collected after one year of litter production under pre-treatment conditions (elevated CO_2: 560 ppm or enhanced N: 3 g m^{-2} y^{-1} NH_4NO_3) and decomposed the following year under treatment conditions (same as pre-treatment). By considering the cross-effects between pre-treatments and treatments, we distinguished between the effects on mass loss of 1) the pre-treatment-induced litter quality and 2) the treatment conditions under which the litters were decomposing. The combination between CO_2 pre-treatment and CO_2 treatment reduced *Polytrichum* decomposition by −24% and this can be explained by litter quality-driven decomposition changes brought by the pre-treatment. CO_2 pre-treatment reduced *Eriophorum* litter quality, although this was not sufficient to predict decomposition. The N addition pre-treatment reduced the decomposition of *Eriophorum*, due to enhanced lignin and soluble phenols concentrations in the initial litter, and reduced litter-driven losses of starch and enhanced litter-driven losses of soluble phenols. While decomposition indices based on initial litter quality provide a broad explanation of quantitative and qualitative decomposition, they can only be taken as first approximations. Indeed, the microbial ATP activity, the litter N loss and resulting litter quality, were strongly altered irrespective of the compounds' initial concentration and by means of processes that occurred independently of the initial litter-qualitative changes. The experimental design was valuable to assess litter- and ecosystem-driven decomposition pathways simultaneously or independently. The ability to separate these two drivers makes it possible to attest the presence of litter-qualitative changes even without any litter biochemical determinations, and shows the screening potential of this approach for future experiments dealing with multiple plant species.

Keywords:
Litter quality and decomposition, Global change, Peatlands, *Sphagnum fallax*, *Eriophorum vaginatum*, *Polytrichum strictum*, Elevated CO_2, Enhanced N deposition, Carbon based secondary compounds, Microbial activity

1. Introduction

Despite covering less than 3% of the Earth's land surface, northern peatlands contain 15–30% of the world's soil organic carbon or between 34 and 46% of the carbon (C) held in the atmosphere (Limpens et al., 2008). When growing, these

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ecosystems constitute an almost continuous carbon sink and as such are a key element in the global C cycle. Any modification to their C accumulation potential could have significant feedback effects on the net increase of atmospheric CO_2—C. Carbon accumulation rates depend on the ratio between net primary production and decomposition, both of which are likely to change under projected higher levels of atmospheric CO_2 and increased N deposition (Wieder, 2006). Particularly in peatlands, the effects of increasing CO_2 and N on the rate of litter decomposition is key to predict the ability of these peat-accumulating ecosystems to act as C sinks in the future. Litter decomposition is driven by two complementary factors both of which are potentially affected by increasing CO_2 and N: 1) litter quality, and 2) microenvironmental conditions.

In numerous ecosystems, global change factors have been shown to alter litter quality, and thus potentially decomposition, via numerous mechanisms. Under nutrient-limited growth conditions elevated atmospheric CO_2 is hypothesised to increase the level of C-based secondary compounds (CBSC: phenolics, terpenes) and/or the non-structural carbohydrates (TNC) in plants (Bryant et al., 1983; Herms and Mattson, 1992; Penuelas and Estiarte, 1998; Estiarte and Peñuelas, 1999). Enhanced CBSC may negatively affect decomposition, whereas the TNC may be easily leached and stimulate microbial activity, and consequently increase decomposition (Melillo et al., 1989; Arp et al., 1997; Norby and Cotrufo, 1998). Increasing N availability through atmospheric deposition has been reported to have different effects on litter decomposition in peatlands, depending on initial litter chemistry, particularly on the content of lignin-like compounds in dominant plant species (Bragazza, 2006). There is also evidence that the availability of labile C compounds like simple sugars can enhance the rate of lignin breakdown by functioning as co-metabolites and that on the contrary N availability can slow down the lignin decay rate (Melillo et al., 1989).

Nevertheless, decomposition is not only a matter of litter production, it is also depending on environmental conditions governing the decomposer's activity, and therefore major changes in microbial communities may occur in soils subject to high atmospheric CO_2 concentration or N inputs regardless of the litter qualities, and these may feedback onto the decomposition efficiency and decomposability of buried litter, but studies on this aspect of global change are rare, especially in natural or semi-natural conditions (e.g. Gilbert et al., 1998; Mitchell et al., 2003; Mitchell and Gilbert, 2004). Another good example of major microenvironmental changes is the indirect atmospheric CO_2 warming effect on peatland-ecosystems which will affect both primary production and ecosystem respiration.

To experimentally separate the effects of CO_2 and N enrichment on the two aforementioned litter decomposition drivers, two conditions are needed: (1) litter should be both produced and decomposed under field-experimental conditions; (2) both litter- and ecosystem-mediated decomposition processes should be combined. Although similar litterbag transplantation approaches (i.e. without factor and control crossings) have been used to separate between litter and microenvironment effects on decomposition (e.g. Belyea, 1996; Trinder et al., 2009), to our knowledge there has not been any attempt to cross-link specific litter production with litter decomposition conditions to study the litter decay in a full-factorial design that includes two phases set under treatment or true control conditions.

For this aim, we set up a field experiment in a cut-over *Sphagnum* peatland, which is representative for large areas of human-impacted peatlands in Central Europe. We chose three dominant species: cottongrass, *Eriophorum vaginatum*, and two moss species, *Sphagnum fallax* and *Polytrichum strictum*. By considering the cross-effects between pre-treatments and treatments, our objective was to distinguish between the effects upon percent mass loss, evolving litter quality and microbial ATP (Adenosine Tri-Phosphate) activity due to: 1) the pre-treatment-induced litter quality and/or 2) the treatment conditions under which the litters were decomposing. We hypothesised that both of these pathways would play an important and quantifiable role in the evolving litter quality, microbial activity and resulting decomposition, and that both pathways are driven by modified nutrient-limited growth and microbial activities brought by elevated CO_2 and enhanced N deposition.

2. Materials and methods

2.1. Study site

The experiment was carried out in a regenerating ombrotrophic *Sphagnum* peatland in the Swiss Jura Mountain, (Chaux-des-Breuleux, 47°15′N, 6°55′E, elevation: 1000 m.a.s.l.) during three consecutive years (year 1: litter production; year 2: litter decomposition). The mire has been drained and the peat exploited until 1945. The vegetation cover is mainly a mosaic assemblage of two dominant mosses, *P. strictum* Menzies ex Bridel and *S. fallax* (Klinggr.) Klinggr, growing intricately among *Eriophorum vaginatum* L. tussocks. The average daily temperature in the warmest and coldest months are 15 °C and −5 °C, respectively. On average, annual precipitation is 1390 mm, the snow-free cover lasts for 245–285 d y^{-1}, and the nitrogen deposition rate was estimated at 15 kg ha^{-1} y^{-1} (Nabel, 1995).

2.2. Experimental set-up

Two 200 m^2 surfaces were selected to set-up two parallel experiments (sub-sites): 1) the CO_2 enrichment experiment: elevated CO_2 (C+: 560 ppm) versus ambient CO_2 (C−: 360 ppm); 2) the N fertilization experiment with N addition (N+: 3 g N m^{-2} y^{-1}) versus ambient N deposition (N−: 1.5 g N m^{-2} y^{-1}). Within each sub-site, five plots (replicates) were randomly selected and attributed to enrichment (C+ or N+) and control (C− or N−), making a total of ten plots in each sub-site.

The sub-site for the CO_2 experiment was equipped with a CO_2 enrichment system composed of five MiniFACE (free air CO_2 enrichment) rings (diameter = 1 m) connected to a gas inlet and computerized control system (Miglietta et al., 2001). The five control rings were not connected to the gas inlet and only air was blown. On the sub-site for the N fertilization experiment, mineral N was applied on four plots (1 × 1.5 m) in six applications during each growing season as a fine spray of an aqueous solution of NH_4NO_3 in 2 l of distilled water per plot and per application. Control plots received 2 l of distilled water.

In each separate experiment, the litters of three plant species produced under pre-treatment or control conditions were collected at the end of the first growing season (year 1). They were then decomposed in the same experiment during the second growing season (year 2) under treatment or control conditions (Fig. 1). Within each experiment, cross-effects between pre-treatment (versus control) and treatment (versus control) were considered in order to establish their respective impact on the litter decomposition process. A replicate unit was composed of paired C+ and C− (or N+ and N−) plots. Litter from each plot (pre-treatment) was decomposed (treatment) in both paired plots.

Three groups of factors influence decomposition: a) the physico-chemical microenvironmental conditions (such as temperature, moisture, light, pH, oxygenation, peat and water chemistry), b) the litter quality before decomposition, and c) the nature and abundance of the living communities (e.g. Belyea, 1996; Heal et al., 1997).

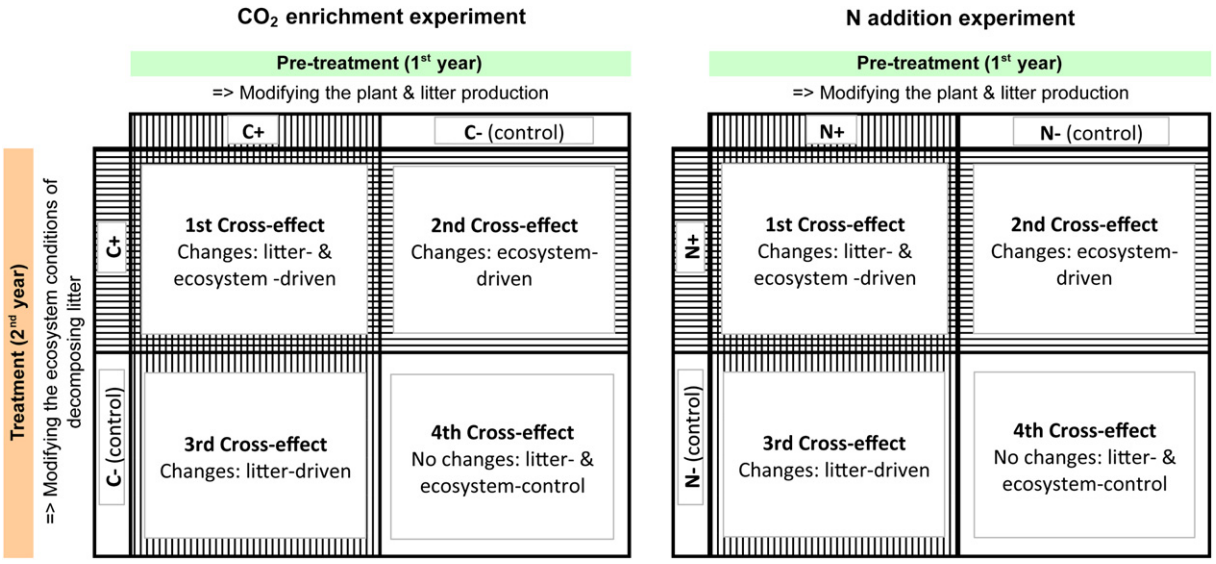

Fig. 1. The experimental set-up where three litter species (*E. vaginatum*, *P. strictum*, *S. fallax*) were collected from plots of two parallel experiments. Within each experiment, and after one year of growth under pre-treatment and its control conditions, the plants were placed in litterbags and inserted into field plots and decomposed under treatment and its control conditions. Cross-effects between the pre-treatment and treatment were considered in order to establish their respective outcome in the decomposition process. A replicate unit (N = 5) was composed of paired C+ and C− (or N+ and N−) plots. Litter from each plot (pre-treatment) was decomposed (treatment) in both paired plots.

In our experimental approach, the pre-treatment acts on factor (b), whereas the treatment influences (a) and (c). Although the combination of pre-treatment and treatment represents the most realistic situation (e.g. C+C+ versus C−C− contrast), our experimental set-up allows us to analyse factor (b) either independently or simultaneously to the two others. The fact that we measured also microbial ATP activity gave us the possibility to differentiate to some extent (a) from (c) in the case of *Eriophorum* litter.

2.3. Litterbags preparation and handling

Our priority was to assess the effects of both the pre-treatment and the treatment on the decomposition process. Standing litter of *Eriophorum*, brown *Polytrichum* parts within an annual growth section, and dried *Sphagnum* plants, all of which had been produced during the first experiment season (pre-treatment, year 1), were collected from the plots in March of year 2. It was assumed that *Eriophorum*'s leaf residence time is less than one year (Vasander, 1982; Jonasson and Chapin, 1985). *Sphagnum* has an unlimited upward growth, which etiolates below the living capitulum and gradually dies and becomes litter. As it was impossible to collect enough pre-treated dead material only from stems and lateral leaves without destroying the experimental plots, we collected the top 5 cm that corresponds to the annual peat-moss growth measured at our sites (Mitchell et al., 2002). The plant material (*Eriophorum*: 6 cm sections, *Sphagnum* and *Polytrichum*: top 5 cm) was dried and placed in 25 μm mesh polyester litterbags (7 × 3.5 cm). This mesh-size excludes meso- and macro-fauna, but allows access to micro-organisms including protozoa and micro-metazoa (Coulson and Butterfield, 1978).

Precisely 0.3 g of dried (40 °C) plant material was placed into each litterbag. The bags openings were then sewn with a polyester thread, the bags were dried and weighed (A, t_0). A total of 120 litterbags (2 experiments × 2 pre-treatments × 2 treatments × 3 species × 5 replicate-plots) were inserted into plots 5 cm below the top of the moss carpet, and left for one season of decomposition under the specific treatments (year 2: March to November). At the end of the field experiment (t_1), the bags were collected, cool-transported to the laboratory, surface cleaned and stored at −80 °C until processing. The bags were then treated for the microbial ATP analyses (see below) and then dried and weighed (B, t_1). The empty bags were also weighed (T).

2.4. Mass loss

Because of the confusion between qualitative and quantitative losses frequently found in literature, we chose to compare the litter mass or chemical data using three different indices:

$$\text{Mass loss (ML, \%)} = \frac{(A-T)-(B-T)}{(A-T)} \times 100 = \sum_{1}^{i} \frac{(C_{iA}-C_{iB})}{(A-T)} \times 100 \quad (1)$$

where mass loss (%) = percentage mass loss; A = litter dry weight with bag before exposure (g); B = litter dry weight with bag after exposure (g); T = emptied litterbag dry weight after exposure (g); C_i = dry weight (g) of an element or chemical compound (i).

To support the description of relative quantitative and relative qualitative losses, we suggest the use of the terms relative decomposition efficiency (RDE) and relative decomposition proficiency (RDP); this by analogy with the terms employed by (Killingbeck, 1996) to describe resorption.

RDE compares the percent mass loss of an element or a chemical compound (i) to the percent mass loss of the total litter (% ML). It functions as a qualitative contribution parameter. Indeed, a compound may have small concentrations and contribute little to average mass loss, although it's decay may be proportionally very high. Thus, a RDE < 1 means relative accumulation throughout the decomposition, while a RDE > 1 means relative loss.

$$\text{Relative Decomposition Efficiency (RDE)} = \frac{\%C_i\text{loss}}{\%\text{ML}} \quad (2)$$

RDP compares the absolute mass loss of an element or chemical compound (i) to the absolute mass loss of the total litter. It allows us to sum up absolute mass losses and functions as a quantitative contribution parameter.

Relative Decomposition Proficiency (RDP)

$$= \frac{C_{iA} - C_{iB}}{(A-T) - (B-T)} = \frac{C_{iA}}{(A-T)} \times \text{RDE} \quad (3)$$

Although, we expressed contents, ratios and losses on a total dry weight basis, in parallel we also expressed these data on a structural dry weight basis, i.e. after subtracting total solubles from the total mass. This increased the significance of results without fundamentally changing their meaning. Non-structural compounds brought high variability among samples but are essential in understanding early decomposition processes including allelochemical effects.

2.5. Biochemical analyses

For the monitoring of functionally relevant litter qualities and decomposition of compounds the following analyses were performed: 1) lignin according to (Morrison, 1972), 2) total solubles (TS) and starch according to (Fales, 1951), 3) soluble phenols according to (Singleton, 1988), 4) total C and total N using an Elemental Analyser (EA1108-CHN, CarloErba, Milano). Due to the low amount of material available, the chemical characterisation of plant litter was carried out only for *E. vaginatum* in initial (t_0) and final (t_1) litter. The dried material was ball-milled and homogenised before analysis. All analyses were duplicated and averaged.

For the general characterisation of decomposers' activity, microbial ATP was measured using a bioluminescence technique (LUMAC-BV, 1987; Simpson et al., 1989). ATP transports chemical energy within cells and is a general indicator of metabolic activity, and is measurable without having to resort to disturbing incubations. The following manipulations were done in a cold room at 5 °C: the frozen litterbags were thawed for 30 min. They were then soaked for 2 min in 50 ml of distilled water and every 30 s the litterbags were turned over. The suspension was filtered through a fibreglass filter of 1.2 μm retention (GF/C, Whatman, Kent). Filtrate subsamples of 1.5 ml were placed in Eppendorf tubes, frozen in liquid nitrogen and stored at −80 °C until analysed. These samples were thawed for precisely 30 min before being analysed. To extract ATP from the microbial cells, 100 μl of NRB® regent was added to 100 μl of sample. After 3 min, 100 μl of LUMIT-PM® (luciferine−luciferase complex with buffer) were added. The mixture was then shortly homogenised and the bioluminescence was immediately measured in relative light units (RLU) with a photometer (Celltester®). To extrapolate microbial [ATP] from emissions a calibration curve was established using ATP standard® solutions.

2.6. Numerical analyses

All variables (expressed on a total and structural dry weight basis) were analysed using linear models (unit, pre-treatment, treatment, pre-treatment × treatment) and on a total or structural dry weight basis. A separate linear model (pre-treatment, unit) was used for *Eriophorum*'s initial litter quality data. In addition, the chemical variables were compared using linear or polynomial regressions, as well as linear correlations (Pearson's r).

To assess the interactions between pre-treatments and treatments, for each response variable we set up one contrast (F-test) within the full cross-effect. Among the four subjacent categories of cross-effects we selected the double amendments versus double controls as contrast (e.g. C+C+ versus C−C−).

Distributions, homogeneity and homeoscedacity of residuals were checked using Q-Q- and scatter-plots for statistical models. The significance level was set at 5%, although we chose to mention *P*-values between 5 and 10% as "marginally significant" as these were very useful in detecting patterns that eventually became significant over time or when the data was expressed on a structural dry weight basis (tested in parallel). For more readability, significant effects, relationships, correlations are mentioned in text without the redundant prefix "significant". The tests were performed using S-plus 4.5 Statistical Software (Insightful Corporation, Seattle, USA).

3. Results

3.1. Decomposition of Eriophorum, Sphagnum and Polytrichum

In general, pre-treatment effects, manifested as alterations of litter quality, were more influential than treatment-induced microenvironmental changes. Irrespective to any pre-treatment, treatment or controls, on average, the mass loss was higher for *Eriophorum* (30%), than for *Polytrichum* (25%) and *Sphagnum* (22%) after 273 days of decomposition.

The CO_2 pre-treatment reduced the mass loss of *Polytrichum* by 18%, whereas *Eriophorum* and *Sphagnum* were unaffected. Moreover, there was no treatment effect on any of the three litter species (Table 1a). Although, there was no overall cross-effect on the mass loss between pre-treatment and treatment, the contrast analysis showed that *Polytrichum* mass loss (C+C+ versus C−C−) was reduced (−24%).

The N pre-treatment reduced the mass loss of *Eriophorum* by 17%, whereas *Sphagnum* and *Polytrichum* remained unaffected (Table 1b). Except for a marginally significant *Sphagnum* decay enhancement (+13%) there was no treatment effect. Although,

Table 1
Effect of elevated CO_2 (C+) or enhanced N (N+) on mass loss (% of initial dry weight) of *Eriophorum vaginatum*, *Sphagnum fallax* and *Polytrichum strictum* litters produced during one growing season (=pre-treatment), and decomposed in the field during the following growing season (=treatment). Pr × Tr = pre-treatment×treatment; %C+or %N+ = percent effect of the treatment versus the control if significant; model based on 20 observations; the Unit effect (df = 4) is not shown but taken into account in the analyses; 2N+/2N− (or 2C+/2C−) = the significant contrast analysis between N+N+ and N−N− (or C+C+and C−C−) litters.

Litter-species	Pre-treatment (df = 1) effects				Treatment (df = 1) effects				Pr × Tr (df = 1)
(A) CO_2 experiment	C+	C−	%C+	F-ratio	C+	C−	%C+	F-ratio	F-ratio
E.vaginatum	30.0	31.7	−	0.9	29.8	31.9	−	1.4	0.4
S. fallax	21.2	22.1	−	0.2	21.0	22.2	−	0.3	0.0
P. strictum	22.5	27.4	−18	7.5*	24.7	25.2	−	0.1	2C+/2C−: −24%*
(B) N experiment	N+	N−	%N+	F-ratio	N+	N-	%N+	F-ratio	F-ratio
E. vaginatum	30.2	36.3	−17	7.9*	32.8	33.7	−	0.2	2N+/2N−: −19%*
S. fallax	22.4	24.5	−	1.8	24.9	22.0	13	3.3°	0.2
P. strictum	21.5	21.6	−	0.0	21.8	21.2	−	0.14	0.6

Significance levels: °: $0.05 < P \leq 0.10$; *: $0.01 < P \leq 0.05$.

there was no overall cross-effect between pre-treatment and treatment, the contrast analysis (N+N+ versus N−N−) showed that *Eriophorum* mass loss was reduced (−19%).

3.2. Eriophorum litter quality and microbial ATP

Before decomposition (t_0), the CO_2 pre-treatment decreased litter quality by reducing the N content of the collected litter (−8%), increasing the C/N ratio (+9%) and also marginally increasing the lignin/N and (lignin + phenols)/N ratios (+12%, +11%, respectively) (Table 2a), which became significant when expressed as percent of structural dry weight.

Under CO_2 enrichment, the quality of the decomposing litter is mainly inherited from the litter-qualitative changes occurring in the litter production. After decomposition (t_1), the CO_2 pre-treatment reduced the N content of the decomposed litter (−11%) and increased C/N (+13%), and marginally increased lignin/N and (lignin + phenols)/N ratios (+14%, +14%, respectively), which became significant when expressed on a structural dry weight basis. By contrast, the treatment had no significant effect on any of the chemical variables (Table 2a). Although, there was no cross-effect between pre-treatment and treatment, the contrast analysis (C+C+ versus C−C−) revealed a significant decrease of N content (−10%) and an increase of the C/N ratio (+13%).

Nearly all chemical compounds were affected by the litter production under N enrichment. Before decomposition ($t0$), the pre-treatment only marginally significantly modified some chemical variables in *Eriophorum* litter before decomposition (Table 2b), but significant differences appeared in lignin, total solubles, phenols, and (lignin + phenols)/N (+6%, +14%, +42% and +13%, respectively) when these were expressed on a structural dry weight basis.

Under N enrichment, the quality of the decomposing litter was affected by both, changes in the litter production and the

Table 2
Effect of elevated CO_2 (C+) or enhanced N (N+) on the chemical composition of *Eriophorum vaginatum* litter produced during one growing season (=pre-treatment), and decomposed in the field during the following growing season (=treatment), before (t_0) and after decomposition (t_1). F-R = F-ratio; Pr×Tr = pre-treatment×treatment; %C + or N+ = percent effect of treatment versus control if significant; model based on 20 observations; the Unit effect (df = 4) is not shown but taken into account in the analyses; NA = not applicable; 2N+/2N− (or 2C+/2C−) = the significant contrast analysis between N+N+ and N−N− (or C+C+and C−C−) litters.

Contents or ratios	Pre-treatment (df = 1) effects				Treatment (df = 1) effects				Pr × Tr (df = 1)
(A) CO_2 experiment	C+	C−	%C+	F-ratio	C+	C−	%C+	F-ratio	F-ratio
1. Concentrations before decomposition (t_0) in percent dry weight									
Carbon (C)	46.9	46.8	–	0.69	NA	NA	NA	NA	NA
Nitrogen (N)	1.22	1.32	−8	9.4*	NA	NA	NA	NA	NA
Lignin	18.3	18.0	–	0.28	NA	NA	NA	NA	NA
Total solubles	10.1	9.6	–	0.31	NA	NA	NA	NA	NA
Starch	8.88	8.47	–	1.51	NA	NA	NA	NA	NA
Phenols	1.22	1.30	–	0.79	NA	NA	NA	NA	NA
C/N	38.8	35.5	9	9.2*	NA	NA	NA	NA	NA
Lignin/N	15.2	13.6	12	4.6°+	NA	NA	NA	NA	NA
(Lignin + Phenols)/N	16.2	14.6	11	4.7°+	NA	NA	NA	NA	NA
2. Concentrations after decomposition (t_1) in percent dry weight									
Carbon (C)	46.6	46.3	–	1.29	46.4	46.4	–	0.03	0.03
Nitrogen (N)	1.27	1.43	−11	9.2**	1.35	1.35	–	0.01	2C+/2C−: −10%*
Lignin	19.4	19.1	–	0.24	19.1	19.5	–	0.34	0.09
Total solubles	7.76	8.9	–	0.93	7.32	9.34	–	2.93	0.22
Starch	8.89	8.44	–	0.42	8.91	8.42	–	0.49	1.2
Phenols	0.82	0.86	–	0.42	0.86	0.82	–	0.35	0.86
C/N	36.9	32.6	13	17**	34.7	34.7	–	0.01	2C+/2C−: 13%*
Lignin/N	15.4	13.5	14	12**	14.4	14.6	–	0.41	1.0
(Lignin + Phenols)/N	16.0	14.1	14	19**	14.9	15.2	–	0.31	1.2
ATP (ng)	5.94	6.03	–	0.35	5.56	6.4	–	0.56	0.22
(B) N experiment	N+	N−	%N+	F-ratio	N+	N-	%N+	F-ratio	F-ratio
1. Concentrations before decomposition (t_0) in percent dry weight									
Carbon (C)	49.1	48.2	2	3.9°+	NA	NA	NA	NA	NA
Nitrogen (N)	1.01	1.05	–	0.41	NA	NA	NA	NA	NA
Lignin	18.0	17.0	6	3.3°+	NA	NA	NA	NA	NA
Total solubles	12.1	10.7	14	3.7°+	NA	NA	NA	NA	NA
Starch	9.56	9.48	–	0.03	NA	NA	NA	NA	NA
Phenols	1.96	1.38	42	3.1°+	NA	NA	NA	NA	NA
C/N	48.0	46.2	–	0.39	NA	NA	NA	NA	NA
Lignin/N	18.0	16.3	–	2.9	NA	NA	NA	NA	NA
(Lignin + Phenols)/N	20.0	17.7	13	3.1°+	NA	NA	NA	NA	NA
2. Concentrations after decomposition (t_1) in percent dry weight									
Carbon (C)	47.5	46.4	–	0.22	47.9	47.3	–	1.2	2.1
Nitrogen (N)	1.38	1.49	−7	6.5*	1.25	1.62	−23	69***	2N+/2N−: −25%*
Lignin	20.8	20.1	4	3.2°+	20.9	20	–	2.0	0.04
Total solubles	8.73	10.1	−14	3.0°	9.18	9.6	–	0.31	3.3°
Starch	9.07	9.16	–	0.24	8.97	9.26	–	2.3	2.9
Phenols	0.97	0.99	–	0.05	1.00	0.96	–	0.2	1.2
C/N	35.7	32.8	9	7.2*	38.9	29.6	31	77***	2N+/2N−: 42%*
Lignin/N	15.6	13.9	12	8.6*	17	12.5	36	56***	2N+/2N−: 55%*
(Lignin + Phenols)/N	16.4	14.6	12	7.4*	17.8	13.1	36	51***	2N+/2N−: 51%*
ATP (ng)	3.86	5.19	–	0.63	3.89	5.16	−25	7.0*	1.1

Significance levels: °: $0.05 < P \leq 0.10$; *: $0.01 < P \leq 0.05$; **: $0.001 < P \leq 0.01$; ***: $P \leq 0.001$; **+: significant when expressed as percent of structural dry weight.**

microenvironmental conditions. After decomposition (t_1), the N pre-treatment reduced the N content of the decomposed litter (−7%) and increased the C/N, lignin/N and (lignin + phenol)/N ratios (+9%, +12%, +12%, respectively) as well as reduced marginally significantly total solubles (−14%). Although lignin was marginally significantly enhanced (+4%), it came out significant when based on structural dry weight. Moreover, the treatment reduced the N content (−23%) and increased the C/N, lignin/N and (lignin + phenol)/N ratios of the decomposed litter (+31%, +36%, and +36%, respectively). In addition, microbial ATP was inhibited by the enhanced N treatment (−25%) (Table 2b). Although, there was no cross-effect between pre-treatment and treatment on any chemical compound, the contrast analysis (N+N+ versus N−N−) revealed a reduced N content and increased C/N, lignin/N and (lignin + phenol)/N (−25%, +42%, +55% and +51%, respectively).

Correlation analyses suggest roughly equally important effects of various litter quality parameters on microbial activity, irrespective to N pre-treatment or control. We found a negative linear relationship between soluble phenols content before decomposition and the logarithm of microbial ATP activity per litterbag after decomposition ($r^2 = 0.454$, $P = 0.033$, Fig. 2). Furthermore, lignin had no relationship with the ATP whereas N did ($r^2 = 0.455$; $P = 0.032$). Consequently, there was a negative relationship between C/N and Log(ATP) ($r^2 = 0.491$; $P = 0.024$), and (lignin + phenol)/N and Log(ATP) ($r^2 = 0.468$; $P = 0.042$).

3.3. Eriophorum decomposition and associated qualitative chemical changes

No significant differences in mass losses, relative decomposition efficiencies (RDE) or relative decomposition proficiencies (RDP) of the chemical variables were found in response to elevated CO_2 during the decomposition process, neither for the pre-treatment, nor for the treatment. Furthermore, no significant cross-effect and contrasts were observed between the pre-treatment and the treatment for any of the chemical variables. Nevertheless, the average RDE of N was 0.85, which indicates N accumulation in proportion to the mass loss (details not shown).

The enhanced N pre-treatment reduced the mass loss of C (−16%). It also reduced starch loss (−11%), increased total solubles loss (+39%) and soluble phenols loss (+20%), when these three were expressed on a structural dry weight basis (Table 3). However, the pre-treatment had no effect on the RDE or RDP of carbon but enhanced the RDE of total solubles (+72%) and soluble phenols (+42%), and similarly enhanced the RDP of total solubles (+74%) and soluble phenols (+96%) (Table 3).

By contrast to the pre-treatment effects, the N treatment strongly enhanced nitrogen mass loss, RDE and RDP (+531%, +395% and +427%, respectively). More specifically, while the control litter immobilised N (−4% loss) the N-treated litter did not (+19% loss) (Table 3). There was an overall cross-effect between pre-treatment and treatment on the total solubles when these were expressed on a structural dry weight basis. The contrast analysis (N+N+ versus N−N−) revealed a decreased C loss (−19%), increased N loss (+180%), and increased RDE of N, total solubles and phenols (+686%, +126% and +39%, respectively) and RDP of N, total solubles and phenols (+1025%, +97% and +91%, respectively).

4. Discussion

4.1. Decomposition of Eriophorum, Sphagnum and Polytrichum

4.1.1. Polytrichum litter produced under elevated CO_2 decomposes less

Lacking roots, mosses cannot exhibit compensatory root-growth for greater nutrient uptake to match a hypothetical enhancement in

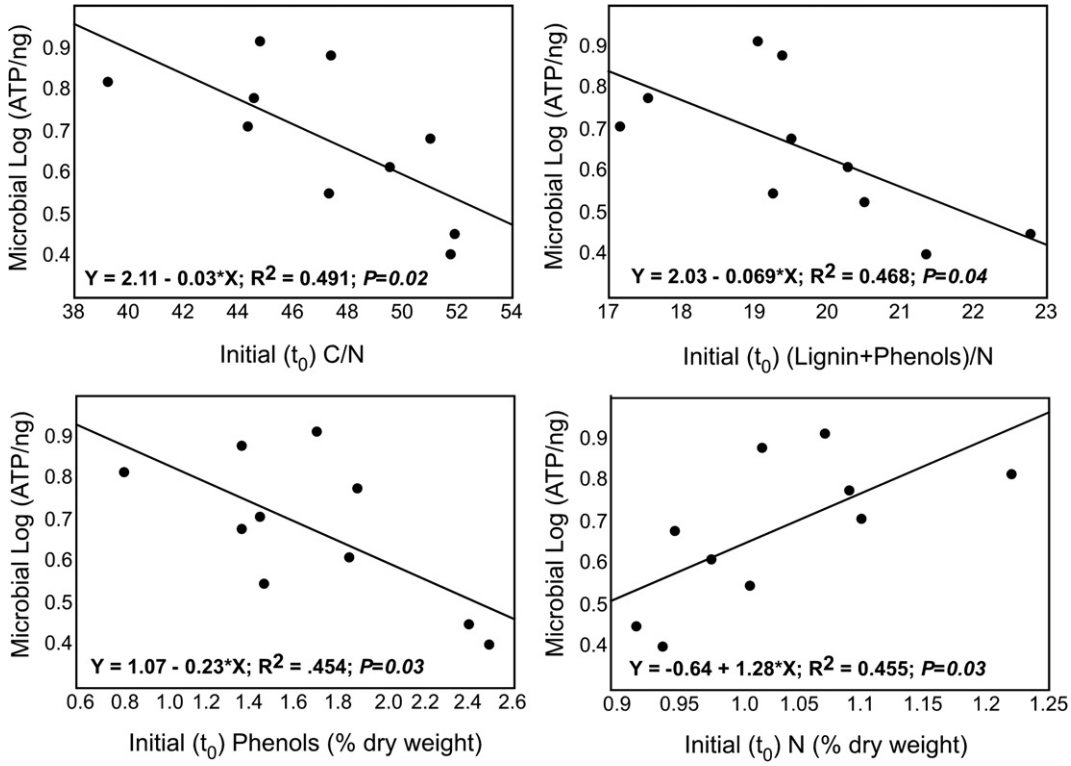

Fig. 2. Linear regression showing significant relationships between initial chemical variables and the microbial ATP activity (in ng per litterbag) found in litter produced in the N experiment. For generalization purposes, the graphs show the overall relationships irrespective to the pre-treatment or control plots (N = 10; R^2 = coefficient of determination).

Table 3
Effect of enhanced N (N+) on losses, relative decomposition efficiency (RDE) and relative decomposition proficiency (RDP) of different compounds/elements found in decaying *Eriophorum vaginatum* litter produced during one growing season (=pre-treatment), and decomposing in the field during the following growing season (=treatment). Pr × Tr = pre-treatment×treatment; %N+ = percent effect of treatment versus control if significant; model based on 20 observations; the Unit effect (df = 4) is not shown but taken into account in the analyses; 2N+/2N− = the significant contrast analysis between N+N+ and N−N− litters.

Variable	Pre-treatment (df = 1) effects				Treatment (df = 1) effects				Pr × Tr (df = 1)
N experiment	N+	N−	%N+	F-ratio	N+	N−	%N+	F-ratio	F-ratio
1. Losses in percent dry weight (% C_i loss)									
Carbon (C)	31	37	−16	5.9*	33	35	–	0.4	2N+/2N− (−19%)*
Nitrogen (N)	4.7	9.7	–	0.9	19	−4.4	531	18**	2N+/2N− (180%)*
Lignin	19	24	–	1.2	19	24	–	1.0	0.1
Total solubles	49	35	39	3.9°+	44	39	–	0.2	3.4°+
Starch	34	38	−11	3.5°+	37	53	–	0.3	0.2
Phenols	64	5.3	20	3.6°+	58	59	–	0.1	1.0
2. Relative Decomposition Efficiency (RDE)									
Carbon (C)	1.0	1.0	–	0.6	1.0	1.0	–	1.0	0.1
Nitrogen (N)	0.15	0.23	–	0.2	0.58	−0.2	395	18**	2N+/2N− (686%)*
Lignin	0.61	0.66	–	0.2	0.57	0.7	–	1.3	0.1
Total solubles	1.6	0.95	72	15**	1.4	1.2	–	0.4	2N+/2N− (126%)*
Starch	1.1	1.1	–	1.6	1.1	1.1	–	1.2	3.1
Phenols	2.1	1.5	42	18**	1.8	1.8	–	0.1	2N+/2N− (39%)*
3. Relative Decomposition Proficiency (RDP)									
Carbon (C)	0.50	0.49	–	1.0	0.49	0.50	–	1.3	1.0
Nitrogen (N)	0.002	0.003	–	0.3	0.006	0.002	427	17**	2N+/2N− (1025%)*
Lignin	0.11	0.11	–	0.0	0.10	0.12	–	1.1	0.1
Total solubles	0.20	0.12	74	13**	0.17	0.16	–	0.0	2N+/2N− (97%)*
Starch	0.11	0.10	–	0.8	0.11	0.10	–	0.7	1.9
Phenols	0.04	0.02	96	19***	0.03	0.03	–	0.0	2N+/2N− (92%)*

Significance levels: °: $0.05 < P \leq 0.10$; *: $0.01 < P \leq 0.05$; **: $0.001 < P \leq 0.01$; ***: $P \leq 0.001$; **+: significant when expressed as percent of structural dry weight.

the photosynthetic rate induced by elevated atmospheric CO_2 concentrations (e.g. Lee et al., 1988; Steinnes et al., 1994; Soares and Pearson, 1997). Therefore a negative pre-treatment effect on the litter quality of both moss species was expected, and hence a reduction in decomposition rates. We observed a negative CO_2 pre-treatment effect on *Polytrichum* decomposition but not for *Sphagnum* (Table 1a). The lack of effect on *Sphagnum* may tentatively be explained by several mechanisms: 1) due to its high porosity and adsorption surface, *Sphagnum* has a greater nutrient relocation capacity than *Polytrichum*, and retains greater amounts of external N, P or K necessary for a balanced growth (Damman, 1978; Malmer, 1988) and may compensate for a nutrient carbon-dilution due to elevated CO_2 conditions. 2) *Sphagnum* host N-fixing bacteria in their hyalocysts, thereby adding extra N to the plant (Rydin and Jeglum, 2006). Under such circumstances, growing *Sphagna* may have had a lower source-to-sink relation than *Polytrichum*, and yielding too little CBSC to experience CBSC-inhibited decomposition. Two parallel studies on the same experimental site provide further evidence for growth-limited responses: the CO_2 enrichment reduced: i) the N content of *Sphagnum* after three years of treatment (Berendse et al., 2001) and ii) the growth of both species, although more negatively for *Polytrichum* compared to *Sphagnum* (Mitchell et al., 2002). Taken together these results suggest that elevated CO_2 indeed affected the metabolism of *Sphagnum* less than that of *Polytrichum*.

The fact that the CO_2 treatment had no effect on the decomposition of any of the three species' litters (Table 1a) suggests that: a) the changes in the physico-chemical environmental conditions did not directly affect decomposition, and/or b) the community structure or activity of micro-organisms was neither directly affected by the treatment nor was it indirectly influenced by a greater rhizodeposition from the vascular plants undergoing the treatment (Fig. 1). However, elevated CO_2 was shown to modify the structure of microbial communities at this study site, but did not affect total microbial biomass (Mitchell et al., 2003). More recently, Kang et al. (2005) showed that enzyme activities involved in N or P mineralisation only increase under elevated CO_2 when nutrient limitation was strongly exerted, while Wolf et al. (2007) showed increased mineralisation of soil organic matter of wetlands.

Although it is important to study and split the relative effects of pre-treatment and treatment on litter decomposition to better understand the processes, these two effects are not separated in nature. With global change, what occurs is the contrast effect (C+C+ versus C−C−), which was found to reduce *Polytrichum* litter decomposition by −24% but not to affect *Sphagnum* decomposition.

4.1.2. Eriophorum litter produced under enhanced N decomposes less

The fact that the N addition pre-treatment decreased *Eriophorum* litter decomposition agrees with the change in reduced litter quality found both before decomposition (t_0) increased lignin and phenols, which were marginally significant but became significant when expressed as percent of total structural dry weight, and after decomposition (decreased N and increased C/N, lignin/N, and lignin + phenols/N). This reduction in litter quality most likely appeared in leaves produced the previous year in late spring when the productivity of *Eriophorum* and the discrepancy between N and further major nutrients availabilities were at their highest. Indeed, in two parallel studies we revealed K-limited growth for these vascular plants under enhanced N deposition (Hoosbeek et al., 2002; Siegenthaler et al. unpublished observations). Thus, while mosses, and especially *Sphagnum*, may have tolerated a greater K-depletion due to translocation capabilities (Malmer, 1988) and slow growth, *Eriophorum* may have become growth-limited at peak production. In principle, vascular plants can increase their root-growth for better nutrient foraging, however their efficiency in P-uptake may not be increased concomitantly (Stulen and Denhertog, 1993; Gressel and McColl, 1997). Besides, we must keep in mind that ontogenic effects alone may contribute to enhancing the production of plant secondary compounds (Herms and Mattson, 1992).

The enhanced *Sphagnum* litter decomposition trend observed under N treatment may indicate that the microenvironment began to have an impact on litter decomposition. Whether this is due to

stimulated microbial activity or due to physico-chemical leaching is arguable. However, in contrary to *Eriophorum* litter, *Sphagnum* litter may be able to retain dissolved N in its porous cells, enabling greater microbial activity. Consequently, response of bogs to global change ought to depend on the relative abundance of these species.

4.2. Eriophorum litter quality

4.2.1. Under CO_2 enrichment, Eriophorum litter has lower N content

The only change that occurred in the CO_2 pre-treated *Eriophorum* litter compounds is the reduction of the N content (Table 2a), with resultant changes in C/N, lignin/N, (lignin + phenols)/N ratios, as found commonly in several studies (e.g. Johnson and Lincoln, 1990; Koch and Mooney, 1996; van der Heijden et al., 2000). Despite the fact that these ratios were enhanced, they were not sufficient to predict the ensuing decomposition since no relationships were found between them and the mass loss. It remains that the lignin/N and (lignin + phenols/N) ratios are more useful than C/N to predict the early stages of decomposition (Melillo et al., 1989; Hirschel et al., 1997) as it specifies the nature of the "C". For example, the "C" hidden in the C/N ratio includes all the carbon-rich compounds such as starch, soluble sugars, hemi-cellulose, cellulose and the CBSC. A high content in easily leached total solubles positively contributes to the litterbag's mass loss (RDE $>$ 1), whereas precisely the opposite occurs with lignin (RDE $<$ 1). Furthermore, as bacteria can readily sequester N from their surroundings, particularly during the early stages of decay, decomposition may depend only slightly upon the litter's initial N content. Consequently the nominator remains the most important variable to be considered.

4.2.2. Under N enrichment, Eriophorum litter has higher carbon contents, and in particular more lignin and phenols

As compared to the CO_2 experiment, in the N experiment, after decomposition, the C/N, lignin/N and (lignin + phenols)/N ratios were similarly enhanced by the pre-treated with the difference that their increase was due to the enhanced carbon-rich contents such as lignin, total solubles or phenols (expressed on structural dry weight basis) together with the reduction in N content (Table 2b). Furthermore, the pre-treatment reduced decomposition and there was a negative linear relationship between the lignin content and % mass loss ($r^2 = 0.415$, $P = 0.045$) and a negative linear relationship trend between the (lignin + phenols)/N ratio and % mass loss ($r^2 = 0.363$, $P = 0.065$). Lignin is the most abundant and recalcitrant aromatic compound found in nature. It is quantitatively important in *Eriophorum* litter, in our case representing on average 18% of the dry weight, and as reported by (Carreiro et al., 2000), may inhibit ligninolytic enzyme activity and decomposition under prolonged N addition. Thus, in contrast to what has been discussed regarding the CO_2 pre-treatment, the above-mentioned ratios can better predict the early stages of the ensuing decomposition, because their nominator was also affected by the pre-treatment.

The real benefits of our experimental set-up become clear if we consider the effect the treatment had on the final litter quality. The treatment very significantly increased all the ratios by about 30–35% (Table 2b). However, these ratio increases are not the result of changes in the decomposition efficiency of easily decomposable compounds (discussed below). The real cause for this common increase is the great N loss, and the lack of net N immobilisation for treated litter. These changes in N dynamics between treated and control litters can only have been triggered by ecosystem-mediated changes including abiotic and biotic factors. This demonstrates the importance of factors not solely driven by the plant in the study of litter decomposition, even more if we consider the significant ensuing contrast effect (N +N+ versus N−N−) on the reduction of the N content. We shall later set out our results in terms of losses and draw parallels with microbial activity.

4.3. Eriophorum decomposition and associated qualitative chemical changes

4.3.1. General comments

Although the lignin content increased during decomposition (RDE $<$ 1), the RDP of lignin was on average just as important as that of total solubles. It follows that small changes in the lignin content, or its intrinsic quality, have a great impact on decomposition in general. Furthermore, no linear correlation was found between lignin loss and either starch or total solubles that would function as decay triggering co-metabolites, nor did a higher litter N content slow the lignin loss down.

4.3.2. The reduction of the litter N content in the CO_2 experiment does not hamper micro-organisms in their N requirements

It is not so surprising to find no CO_2 effect on the decomposition indices (% mass loss, RDE and RDP) of the pre-treated litter compounds, because as mentioned above, even if the N content was reduced under the pre-treatment (Table 2a), it may not be as relevant as lignin or soluble phenols in the decomposition process, and a reduction in the N content alone probably is not a sufficient condition to reduce the rate of litter decomposition. In fact, we found no relationship between the initial N content and percentage mass loss. This lack of relationship agrees with the fact that micro-organisms may easily immobilise N from their surrounding environment to satisfy their N requirement (Damman, 1988). In our case, even if no net immobilisation was observed in the litter, the RDE of N was $<$1 and clearly indicates that N was accumulating in proportion to the other compounds of the residual litter during decomposition and that external N inputs were present to counterbalance N losses.

4.3.3. Eriophorum litter produced under N enrichment releases more phenols and hampers microbial activity

Because N pre-treated litter decomposed less, and because the sum of the compounds' RDP cannot cover the total mass loss, the enhanced losses in soluble compounds of pre-treated litters (Table 3) must have been over-compensated by reduced losses in starch together with unmeasured recalcitrant structural compounds other than CBSC. These undefined recalcitrant compounds may be hemicellulose, tannins, and to a lesser extent, cellulose. According to Penuelas and Estiarte (1998), an enhanced source-to-sink relation would enhance the production of CBSC or the structural compounds such as cellulose and hemi-cellulose in most vascular plants. N addition could also increase the production of N-based secondary metabolites, and conceivably hamper decomposition (Herms and Mattson, 1992).

Since the lignin RDP was not affected in pre-treated litter, the higher initial lignin content was responsible for the greater lignin content and ratios found after the decomposition process. By contrast, the pre-treatment enhance the soluble phenols content of the standing litter (42%) and this was also accompanied with a higher loss (+20%), higher decomposition efficiency (RDE: +42%) and a higher contribution to mass loss (RDP: +96%) of soluble phenols (Table 3). Although smaller in content, soluble phenols lost on average 59% of their mass during the decomposition process and most probably influenced microbial activity. Phenols have indeed been found to hinder N availability by inhibiting enzyme activity and slowing down the release of N from the peat, and can also have toxic allelochemical effects on other living organisms (Palm and Rowland, 1997). We found a significant negative linear relationship between the soluble phenols content before decomposition

and the logarithm of microbial ATP activity per litterbag after decomposition (Fig. 2). Furthermore, lignin had no relationship with the ATP whereas N did. It follows that the negative relationships found between C/N and Log(ATP), and (lignin + phenol)/N and Log(ATP), could principally be attributed to the increase in phenols and, to a lesser extent, the slight decrease in the N content. Finally, the significant increases observed in the RDE and RDP of total solubles and soluble phenols could be the result of chemical changes within the intrinsic nature of these generic compounds that would not be captured with rather unspecific biochemical analyses.

Although, we observed no significant treatment-induced decomposition changes, except for N. It is interesting to see that the nitrogen treated litter did not immobilise N as did the control litter (Table 3). Net immobilisation (% N loss < 0) is a standard microbial process that takes place in the early stage of decomposition. Many litter types incubated in different temperate ecosystems showed that after a short initial period of leaching (1–2 months), litter immobilised N for 1–2 years, then began a period of net N release (Hobbie and Chapin, 1996). Among the treated litterbags, the enhanced N loss during the decomposition process was concomitant with a significant reduction in microbial activity. However no significant relationship was found to exist between these two variables.

Additionally, we estimated the microbial N requirement by calculating the % difference between the actual $N(t_1)$ after decomposition, and an inferred $N'(t_1)$ resulting from a decrease of the initial $N(t_0)$ proportional to the C loss. Hence, since neither N requirement nor N loss were linked to microbial activity, we presume that the reduction in microbial activity among the N-treated litterbags may have been induced by the direct toxic effects of NH_4^+ deposition (Pearson and Stewart, 1993).

4.4. Conclusions

Global change can potentially influence decomposition both through effects on litter quality and through changes in the soil environment (biotic and abiotic). Our experimental approach allowed us to test these two possible pathways simultaneously or independently.

We explored the general hypothesis that the pre-treatment may affect litter quality, which itself acts upon decomposition, and which in turn affects N availability and feeds back to plant growth and community structures. By considering the treatment effects – thus excluding the litter quality effects – we have made a step in another direction: the alternate general hypothesis which states that the treatment could change the conditions for growth, root allocation and/or rhizodeposition, which would modify microbial biomass and nutrient availability to plants (Diaz et al., 1993; Norby and Cotrufo, 1998; van Ginkel et al., 2000).

Our results emphasize that while decomposition indices based on initial litter quality provide a broad explanation of the quantitative and qualitative decomposition, they can only be taken as first approximations. Indeed, not only did one or the other pre-treatment (elevated CO_2 or enhanced N) affect the mass loss of litter and/or various chemical compounds, but the microbial activity, the litter N loss, and the resulting litter quality, were strongly altered irrespective of the compounds' initial concentration, by means of processes that occurred independently of the initial litter-qualitative changes. The outcome of these important ecosystem-driven changes is not clear, as these were indeed linked to the microbial activity but not explicitly to the mass loss. We hypothesise that these ecosystem-enhanced N losses occurring under N fertilization would hinder decomposition over a longer period.

In addition, even without biochemical determinations, we can state that changes in the initial litter quality of *Polytrichum* must undoubtedly have occurred since the ensuing decomposition was affected by the CO_2 pre-treatment. Testing the pre-treatment effect on control decomposition depicts the imprint of the plant's intrinsic response. Hence, although we did not analyse these litter quality changes for mosses, they were implicitly revealed from that imprint. This shows the screening potential of the experimental design, especially in experiments that deal with multiple plant species.

Finally, this experimental set-up appears to be valuable to assess which of litter- or ecosystem-driven mechanism exert the strongest control on the decomposition process, or indeed if they both play a significant role which could even be opposite and resulting in no overall effect. Yet, the key next steps in future research will be to combine the CO_2 and N enrichments, as well as include major effects such as global warming, using the same design.

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