Effects of Elevated Atmospheric CO_2 and Mineral Nitrogen Deposition on Litter Quality, Bioleaching and Decomposition in A Sphagnum Peat Bog.

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Abstract: A brief overview of an attempt to link the effect of elevated CO_2 and nitrogen deposition on litter quality and decomposition in a *Sphagnum* peat bog is given. Litter of three common species (*Eriophorum vaginatum*, *Polytrichum strictum* and *Sphagnum fallax*) was collected from field plots after two years of pre-treatment in two parallel experiments: a) Elevated atmospheric CO_2 experiment, b) mineral nitrogen fertilisation experiment. The litters were put into litterbags, leached and inserted into field plots for 3 months, where they decomposed under specific treatment. Distinction between effects of initial litter quality and decomposition on mass loss in the bioleaching and/or in field decomposition process could be tested using a particular set-up in which cross-effects of pre-treatment and treatment were considered.

1. INTRODUCTION

Twenty times more CO_2-C is cycled annually along the terrestrial photosynthesis-decomposition pathway than the annual net addition to the atmosphere. Small changes in net primary productivity or in decomposition of soil organic carbon (SOC) could significantly influence the net increase of atmospheric CO_2. Because elevated CO_2 may strongly influence (1) the net primary productivity and specific composition of natural vegetation, and (2) the chemical composition of plant material, and therefore the decomposability of plant litter, strong feedback into the SOC pools are to be

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expected [1]. Peatlands contain 20-30 % of the world's soil organic carbon (SOC) [2]. If growing, they constitute an almost continuous carbon sink and are therefore a key element of the carbon cycle. Any modification to their functioning could have significant feedback effects on global warming.

Under growth limiting conditions (nutrients, water), and according to the carbon-nutrient balance hypothesis, elevated atmospheric CO_2 would either directly or indirectly increase carbon based secondary or structural compounds (CBSSC) as well as total non-structural carbohydrates (TNC) of growing plants [3].

The opposite effect could be expected for higher mineral nitrogen deposition. These direct or indirect hypotheses were extended and referred to by [4] as a 'carbon supply model of secondary plant metabolism' or as the 'amino acid diversion model of secondary plant metabolism', respectively. There are few answers to how these changes in living plants affect the litter quality and the ensuing decomposition process [5]. We therefore set up an experiment to try to link litter quality and decomposition aspects in a Sphagnum peat bog in a perspective of climate change.

Litter of three common species (*Eriophorum vaginatum*, *Polytrichum strictum* and *Sphagnum fallax*) was collected from field plots after two years of pre-treatment in two parallel experiments: a) Elevated atmospheric CO_2 (560 ppm) / ambient CO_2 (360 ppm), b) mineral nitrogen fertilisation experiment: 30 [kg/ha/a] NH_4NO_3 / 0 (control). The litters were put into litterbags, leached under artificial rain and inserted into field plots for 3 months, where they decomposed under a specific treatment.

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2. METHODS

2.1 Study site

The field experiment has taken place in an ombrotrophic peat bog in La Chaux-des-Breuleux, in the Swiss Jura, (47°15'N, 6°55'E, alt: 1000 m) from 27.3.1998 to 1.9.1998. The mire was drained and the peat was exploited until the end of Word war II. The mean daily temperature in the warmest month is 15°C and -5°C in the coldest month. Annual precipitation is 1390 mm and snow covers the site 80 to 120 days a year. Nitrogen deposition is 10-30 [kg/ha/a]. The vegetation is dominated by *Eriophorum vaginatum, Carex nigra, Vaccinium oxycoccos.* The dominant mosses are *Sphagnum fallax, Polytrichum strictum, Aulacomnium palustre* [6].

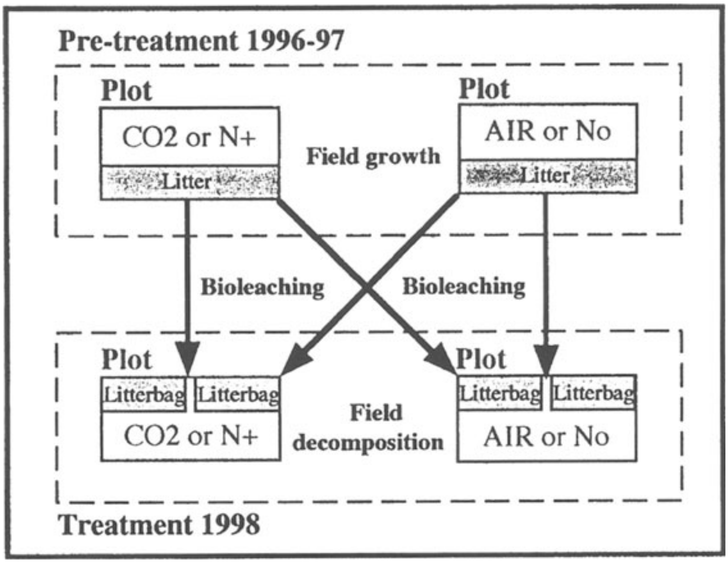

Figure 1: experimental set-up showing a) cross-effects between pre-treatments and treatments, b) the bioleaching and field decomposition processes. One unit is composed of a (pre-)treatment and its control. (Pre-)treatment were assigned randomly to a plot.

2.2 Experimental set-up

Litter from *Eriophorum vaginatum*, *Polytrichum strictum* and*Sphagnum fallax* plants produced during the previous two seasons of pre-treatment (1996 and 1997) were collected from the plots. About 0.3 g of litter material were put into 300 polyester litterbags (25 µm mesh) [7, 8, 9], briefly autoclaved at 110 °C and then exposed to seven cycles of: 1) 12 hours artificial rain (fine spray of distilled water, 6000 mm/12h), 2) 12 hours thawing at –20°C and 3) 10 hours drying at 65°C in a pulsed-air oven. The bags were weighed after each cycle until their dry weight stabilised. The litterbags were leached before being reinserted in the peat surface for the following purposes: 1) To standardise the litter before a short-term field experiment [7]. 2) Together with pre-treatment and/or treatment, to gain a better idea of the qualitative or quantitative contributions of either bioleaching or active decomposition processes on the overall litter degradation. Finally, they were inserted into the field plots for decomposition under particular treatment for a period of three months. At the end of the season, the bags were retrieved, cleaned carefully and dried to constant weight at 65°C. By considering cross-effects between pre-treatments and treatments we could distinguish between the effects of initial litter quality and decomposition on mass loss during the field and the overall decomposition processes. It was also possible to determine the effect of initial litter quality on mass loss during the bioleaching process. Every combination of pre-treatment and treatment effects was replicated: a) three times for *Eriophorum* in the CO_2 enrichment bloc and one time in the nitrogen fertilisation bloc, b) three times for *Polytrichum*, c) two times for *Sphagnum* (Fig. 1).

2.3 Pre-treatments and treatments

The site was divided into two experimental blocs, with no cross-effects between them. The first bloc is equipped with a MiniFace (Free Air Carbon dioxide Enrichment) system [10]. On the second bloc, NH_4NO_3 was finely sprayed every third week on five one metre-squared plots (six applications in total). Each dose was given in two litres of distilled water per plot. Control plots received two litres of distilled water. The treatments were randomly assigned to the plots and there were five replicates per treatment (4 treatments x 5 replicates = 20 plots).a) Atmospheric CO_2 enrichment experiment: CO_2= 560 ppm CO_2 / AIR = ambient (360 ppm CO_2) b) Mineral nitrogen fertilisation experiment: N+=30 [kg/ha/yr] NH_4NO_3 / N_0 = 0 [kg/ha/yr] (control).

2.4 Mass loss

The bags were dried to constant weight at 65°C and put in a desiccator while cooling [11].

The data was expressed and compared using three different ratios. (ML: mass loss; A: litter dry weight with bag before exposure /g; B: litter dry weight with bag after exposure /g; T: emptied litter bag dry weight after exposure /g; C_i: compound dry weight /g)

$$\%\,Total\ ML = \frac{(A-T)-(B-T)}{(A-T)} * 100 = \sum_{1}^{i} \frac{(C_{iA} - C_{iB})}{(A-T)} * 100$$

$$\%\,ML\,ratio = \frac{\%\,C_i\,ML}{\%\,Total\ ML}$$

$$C_i\ contribution\,to\ ML = \frac{C_{iA} - C_{iB}}{(A-T)-(B-T)}$$

2.5 Chemical analyses

Eriophorum vaginatum litter was analysed initially (t_0, 12 samples), after bioleaching (t_1, 20 samples) and after field experiment (t_2, 40 samples).

The 65°C oven-dried material was pulverised through a 0.2 mm mesh grid and homogenised. All analyses were colorimetric and duplicated except for the lignin and total solubles. Mean light extinction values were taken to calculate the concentrations on a percentage dry weight basis. The following analyses were done: a) quantification of lignin in Monocotyledons according to Morrison [12], b) determination of total solubles, soluble sugars and starch according to Fales [13, 14], c) determination of soluble phenols according to Singleton [15], d) determination of total C and total N using CarloErba Elemental Analysor EA1108 CHN, e) determination of bacterial ATP activity using bioluminescence (1997) [16], f) PH determination by titration on gravity water from the first 10 cm of the moss carpet.

2.6 Statistical analyses

Distribution and normality of variables were first checked using box plots and histograms. Those containing too many and/or too distant out layers were analysed using ranked values. Variables were analysed separately using an additional three-way (pre-treatment, treatment, unit, pre-treatment x treatment) ANOVA model. The analyses were done separately for bioleaching, field and overall processes. To perform these ANOVAs we used the S-PLUS (1995) program package. The significance level was set at 5%, but probabilities between 5% and 10 % were considered worth mentioning in the text as being 'almost significant'.

3. RESULTS

3.1 Species specific decomposition

Elevated CO_2 pre-treatment significantly increased % total ML of *Eriophorum vaginatum*, (9 %, $P<0.001$) significantly decreased that of *Polytrichum strictum* (-7 %, $P<0.05$) and had no significant effect on *Sphagnum fallax* in the bioleaching process. However, in the field decomposition process, the pre-treatment did not significantly affect *Eriophorum vaginatum* and *Sphagnum fallax* but had a strong negative effect on *Polytrichum strictum* (-67 %, $P<0.0001$) % total ML. As a result, the pre-treatment had a significant positive effect on the overall % total ML of

Eriophorum vaginatum (10 %, $P<0.05$) and a significant negative effect on *Polytrichum strictum.* (-15 %, $P<0.0001$).

Elevated CO_2 treatment significantly increased % total ML in the field decomposition and overall processes for *Eriophorum vaginatum* (45 %, $P<0.05$, 12, $P<0.05$, respectively) but had no effect on the other two species (Fig. 2). Nitrogen pre-treatment had no effect on % total ML in the bioleaching process. However, in the field decomposition process, there was a negative trend which was almost significant for *Eriophorum vaginatum* (-41 %, $0.05=P<0.1$). All treated litters lost less mass in the field and the overall processes in spite of not being significant. This was not the case in the bioleaching process for *Eriophorum vaginatum* and *Sphagnum fallax*, which lost more mass (n.s.). In the cases of *sphagnum fallax* and *Polytrichum strictum* the treatment had an almost significant negative effect on % total ML in the field decomposition process (-60 %, -63 %, $0.05=P<0.1$, respectively).

3.2 Litter quality

Remark: soluble sugar concentrations were on average <1 % dry weight and were therefore neglected. Expressing the concentrations and losses on a structural dry weight basis did not alter the results.

Elevated CO_2 pre-treatment did not significantly affect the initial litter quality of *Eriophorum vaginatum.* The higher almost significant C-to-N and Lignin-to-N ratios in pre-treated plots were mainly due to a 13 % lower almost significant N concentration rather than to an increase in C. Secondary compounds like lignin and soluble phenols were almost significantly lower (7 % and 9 % respectively) in pre-treated litter. Starch concentration was almost significantly enhanced by 8 %. The total solubles were 6 % lower in elevated CO_2 plots (n.s.).

Due to a limited amount of litter material in some plots of the nitrogen fertilisation bloc, the initial litter of the five replicates for pre-treatment and control had to be pooled. The differences in initial concentrations could not be tested. However, the results remained very informative. Lignin and soluble phenols content were enhanced by the pre-treatment by 7 % and 87 % respectively. However, the C-to-N and L-to-N ratios were slightly lower because of lower C and higher N concentrations in pre-treated litter.

3.3 Qualitative bioleaching and decomposition

The higher (9 %, $P<0.001$) % total ML for CO_2 pre-treated litter in the bioleaching process could only be explained (linear regression, $R^2=0.492$, $P=0.0238$) by a higher almost significant C loss, yet neither the starch loss nor total solubles loss could account for it. However, in the field decomposition process, there was a 766 % higher but not significant starch loss which could over-balance the significant 32 % decrease in total solubles loss, and together with an enhanced N loss (42 %, n.s.) match the positive not significant % total ML.

Lignin, together with the phenol loss (both enhanced by the treatment), accounted for more than half of the enhanced litter loss in the field process. This wasn't the case for N, which lost (121 %, n.s.) less in the treated plots. As in the pre-treatment, the total solubles loss was significantly reduced by treatment (-74%, n.s.).

The bioleaching process was not affected by the nitrogen fertilisation pre-treatment in terms of % total ML. In the field decomposition process, there was an almost significant decrease (-41%) of % total ML coupled with a general negative effect on all compounds loss except for C and soluble phenols. One part of this reduced % total ML could be attributed to a net immobilisation of nitrogen (95 %) and total solubles (107 %) which were greater in the pre-treated plots. The pre-treatment tripled the soluble phenols loss significantly ($P<0.001$) which represent 46 % of the initial soluble phenols dry weight and contributed to 17 % of % total ML (only 4 % in the control).

The treatment decreased the % total ML in the field decomposition and overall processes (-70 %, n.s., -9 %, n.s.). However, the carbon loss was almost significantly decreased (-12 %) and all the other compounds followed the same trend. An enhanced (43 %) starch loss was the only exception found, although was not significant.

3.4 Bacterial ATP activity and PH

The 1997 experiment showed that nitrogen treatment significantly decreased the bacterial ATP activity in the litterbags and slightly decrease the gravity water PH (-25%, $P<0.05$, -2%, n.s., respectively).

4. DISCUSSION

4.1 Species-specific decomposition

Eriophorum vaginatum and *Polytrichum strictum* reacted in the opposite way to CO_2 pre-treatment and treatment during both processes. Pre-treated *Sphagnum fallax* only reacted similarly to *Polytrichum strictum* during the field decomposition process.

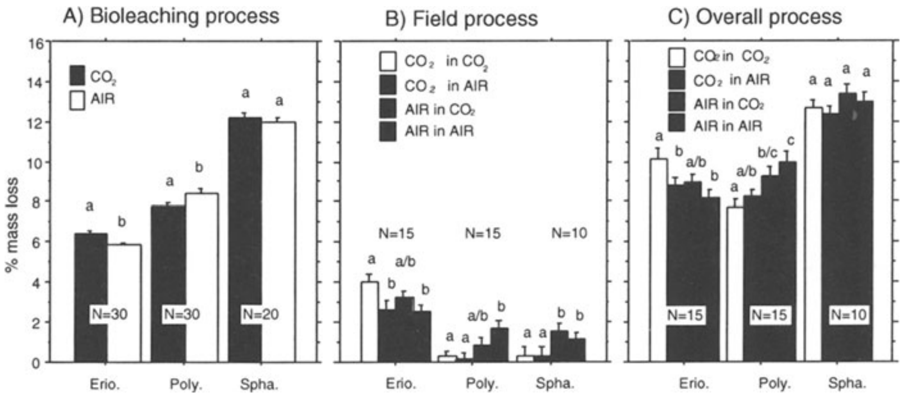

Figure 2. Species-specific effects of pre-treatment and treatment on litter mass loss (values followed by different letters are significantly different (P<0.05). Error bars indicate SEM. N represent the number of bags used for each nominal variable).

This may emphasise disparities in the physiology between vascular plants and mosses. While lignin and soluble phenols concentrations were not enhanced in elevated CO_2 pre-treated *Eriophorum vaginatum* litter, starch concentration was almost significantly increased. This accumulation of starch could be due to an 'energy overflow' from enhanced rate of photosynthesis [4]. Hence, the higher % total ML for pre-treated *Eriophorum vaginatum* litter could be explained by a high contribution of starch and N rich compounds (amino-acids or proteins) to the litter ML in the field decomposition process. It seems that N was not limiting growth enough to increase either directly or indirectly CBSSC. However, mosses (*Sphagnum* and *Polytrichum)* which have no root system, rely more on nitrogen deposition for their N nutrition than do vascular plants which can extend their root system for greater uptake [17, 18]. Therefore, nitrogen could become a strong limiting factor for these plants under elevated CO_2 conditions. Under limited growth and in accordance with the carbon-nutrient balance hypothesis [3, 4, 19], more secondary compounds could be produced. If these compounds happen to be easily leachable secondary compounds such as soluble phenols, they may inhibit microorganisms' activity and active decomposition in the field process. This could explain the negative effect of CO2 treatment on *Polytrichum strictum* ML in the field decomposition. These remain assumptions, and require further investigation.

4.2 Lignin and decomposition

The fact that lignin contribution to the litter ML was on average 60 % higher in the field than in the bioleaching process could show that even in the relatively short period of field decomposition lignin had already been selectively degraded by microorganisms or soil enzymes. It implies that early colonisers may as well be lignolytic fungi or bacteria. Fungi and bacteria were indeed shown to be the dominant groups of microorganisms in our experimental site (34 % and 50% of total microbial biomass respectively) [20]. No taxonomical and enzymatical studies have been undertaken for these two groups. According to [21] principally responsible for lignocellulose degradation are aerobic filamentous fungi, and the most rapid degraders in this group are Basidiomycetes. Furthermore, the lignin contribution to the *Eriophorum vaginatum* litter ML during field decomposition was lower in nitrogen pre-treated and treated plots as compared to control albeit not significant. This may as well reflect a inhibition of microbial activity.

4.3 Inhibition of decomposition

Despite the fact that these results can not be tested, the fact that nitrogen pre-treated litter contained 6 % more lignin and 86 % more soluble phenols should retain our attention. This could reflect a nutrient limited growth and could match with the carbon balance hypothesis. Since the TNC were not enhanced together with lignin and soluble phenols, the 'amino-acid diversion model of secondary plant metabolism' which doesn't consider increased levels of TNC as the major trigger, could be favoured.

This hypothesis rather states that increased accumulation of phenolics stems from a decrease use of a common precursor (phenylalanine or tyrosine) for protein synthesis [4]. Higher nitrogen deposition, even using NH_4NO_3, might decrease PH [22]. This acidification could in turn decrease exchangeable cations (Ca^{2+}, Mg^{2+}), increase NH_4^+ and NO_3^- leaching, and decrease decomposition [23, 24]. All these effects would lead to a nutrient limited growth. Our experiment has even shown that the pre-treatment significantly ($P<0.0001$) increased the soluble phenols loss in the overall process (46 % of their own weight has been lost). Results from a previous experiment in 1997, made with litter that did not undergo bioleaching, have indicated that the bags' bacterial ATP activity decreased with the same amount of NH_4NO_3 used.

4.4 Quantitative versus qualitative mass loss

In the case of *Eriophorum vaginatum*, the bioleaching ML and the field decomposition ML were both compared to the overall ML (Fig. 3).

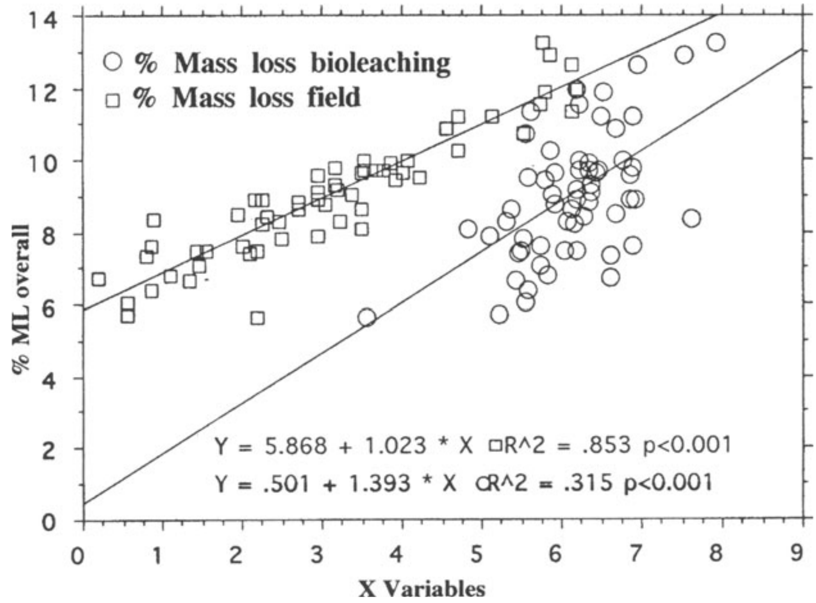

Figure 3: Linear regression for the comparison between a) overall % ML and Bioleaching (n=60)% ML, b) Overall % and filed (n=60)% ML. Example taken for Eriophorum vaginatum litter of the CO2 enrichment bloc.

The comparisons show that: a) bioleaching is quantitatively more important (higher regression coefficient), b) field decomposition is establishing differences between (pre-) treatments and therefore qualitatively more important (higher R^2) for the pattern of the overall ML.

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