Atmospheric nitrogen deposition promotes carbon loss from peat bogs

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Peat bogs have historically represented exceptional carbon (C) sinks because of their extremely low decomposition rates and consequent accumulation of plant remnants as peat. Among the factors favoring that peat accumulation, a major role is played by the chemical quality of plant litter itself, which is poor in nutrients and characterized by polyphenols with a strong inhibitory effect on microbial breakdown. Because bogs receive their nutrient supply solely from atmospheric deposition, the global increase of atmospheric nitrogen (N) inputs as a consequence of human activities could potentially alter the litter chemistry with important, but still unknown, effects on their C balance. Here we present data showing the decomposition rates of recently formed litter peat samples collected in nine European countries under a natural gradient of atmospheric N deposition from ≈0.2 to 2 g·m^{-2}·yr^{-1}. We found that enhanced decomposition rates for material accumulated under higher atmospheric N supplies resulted in higher carbon dioxide (CO_2) emissions and dissolved organic carbon release. The increased N availability favored microbial decomposition (*i*) by removing N constraints on microbial metabolism and (*ii*) through a chemical amelioration of litter peat quality with a positive feedback on microbial enzymatic activity. Although some uncertainty remains about whether decay-resistant *Sphagnum* will continue to dominate litter peat, our data indicate that, even without such changes, increased N deposition poses a serious risk to our valuable peatland C sinks.

decomposition | global change | litter peat | CO_2

Peatlands cover 2–3% of the land's surface, store approximately one-third of all soil carbon (C) (390–455 Pg), and currently act as sinks for atmospheric C (1, 2). The ability of peatlands to sequester atmospheric C resides in the long-term accumulation of partially decomposed organic matter (i.e., peat). Indeed, acidic water conditions, low soil temperature, frequent waterlogging, and low nutrient quality of plant litter impair decomposition of plant litter, favoring its accumulation (3). In peatlands exclusively fed by atmospheric deposition (i.e., bogs) (1), the accumulated peat is dominated by the remnants of the mosses of the genus *Sphagnum*, which produce a litter poor in nutrients and highly enriched in organochemical compounds such as uronic acids (4) and polyphenols (5) with a strong inhibitory effect on microbial activity and vascular plants (3). As such, *Sphagnum* plants form the bulk of living and dead biomass in bog ecosystems (3).

Because of the strict dependence of bogs on atmospheric deposition as a source of nutrients (1), the increasing availability of biologically reactive nitrogen (N) from industrial and agricultural activities (6) could potentially alter the chemical quality of plant litter with consequent effects on the amount of C released during litter decomposition. Accordingly, an understanding of the mechanisms of bog soil C response to changing N availability is essential for assessing the capacity of peat bogs to continue to contribute to the net land C sink (2, 7).

To investigate the specific role of N availability in litter peat decomposition, we incubated *Sphagnum* litter samples in the laboratory under uniform temperature conditions, after the addition to each litter peat sample of an identical microbial inoculum. These controlled incubation conditions ensured that any difference in decomposition was purely the result of initial peat quality. To avoid confusion with short-term N fertilization effects, we collected litter peat samples under a natural gradient of chronic N depositions, selecting 12 bogs from nine European countries spanning a range from ≈0.2 to 2 g of N·m^{-2}·yr^{-1}. Differences in litter peat chemistry along the gradient of atmospheric N deposition were assessed, determining total N, C, phosphorus (P), and polyphenol concentrations. The amount of C released during litter peat decomposition was assessed by measuring carbon dioxide (CO_2) emission and dissolved organic carbon (DOC) concentration in association with the activity of the main hydrolase and oxidase enzymes.

Results and Discussion

Atmospheric N deposition at each bog correlated positively with the mean N/P ratio ($r = 0.79$, $P < 0.01$; $n = 12$) but negatively with the mean C/N ratio ($r = -0.71$, $P < 0.01$; $n = 12$) in the litter, indicating that chronically increased N inputs affect litter peat chemistry through a higher accumulation of N compared with P and C. Higher N concentrations in litter peat stimulated short-term decomposition as supported by increasing trends of CO_2 emission (Fig. 1). We propose a direct and an indirect effect of increased N availability on litter peat decomposition to be invoked. The direct effect is due to alleviated N limitations on microbial metabolism, which is then stimulated by decreasing C/N ratios of litter peat (8–10). The above mechanism is supported by our observation of decreased activity of chitinase, an enzyme associated with degradation and release of N from organic matter, along the gradient of N deposition (Fig. 2).

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Abbreviations: DOC, dissolved organic carbon; MUF, methylumbelliferyl.

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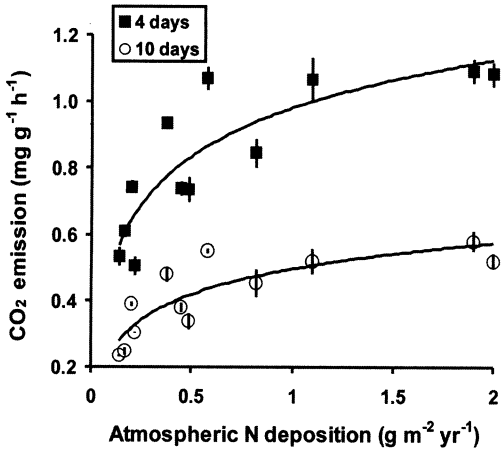

Fig. 1. Hourly CO_2 emission from litter peat samples after 4 and 10 days of incubation in relation to atmospheric N deposition in study bogs. Relationships were explained by a logarithmic regression for both incubation periods [$y = 0.98 + 0.21ln(x)$, $R^2 = 0.75$, $P < 0.01$ and $y = 0.49 + 0.11ln(x)$, $R^2 = 0.73$, $P < 0.01$, respectively]. Each value is the mean (± 1 SE) of three to six litter peat samples.

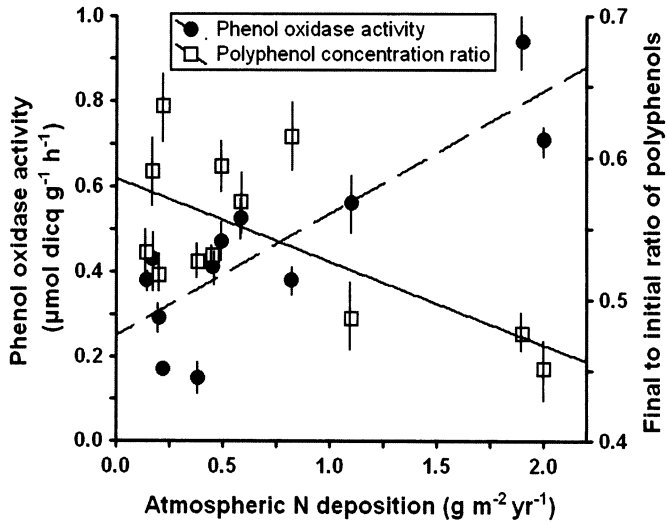

Fig. 3. Increasing N deposition was accompanied by an increasing activity of phenol oxidase ($y = 0.25 + 0.28x$, $R^2 = 0.71$, $P < 0.01$; $n = 12$) and by a decreasing trend of the ratio between mean concentration of polyphenols released at the end and at the beginning of incubation ($y = 0.58 - 0.06x$, $R^2 = 0.43$, $P = 0.02$; $n = 12$). Each value is the mean of three to six litter peat samples (± 1 SE). A negative correlation was found between the activity of phenol oxidase and the final to initial ratio of polyphenol concentration ($r = 0.59$, $P = 0.04$; $n = 12$). dicq, 2,3-Dihydroindole-5,6-quinone-2-carboxylate.

Indeed, because the activity of an extracellular enzyme is regulated by the availability of the nutrient that the enzyme mineralizes (11–13), the decreasing activity of chitinase with increasing N concentration in litter peat supports our view that we are observing a reduction of N limitation on microbial metabolism.

The indirect effect of N availability is related to the negative relationship between initial polyphenol concentration in litter peat and atmospheric N deposition ($y = 1.17 - 0.09x$; $R^2 = 0.58$; $P < 0.01$; $n = 12$). A lower release of polyphenols from litter peat samples collected under higher atmospheric N deposition (i.e., from litter with a lower C/N ratio) reflects a lower production of polyphenols by living *Sphagnum* plants when N availability is high, in accordance with an inverse relationship between rates of protein and polyphenol synthesis (14). Because polyphenols play a pivotal role in inhibiting microbial enzymatic activity (15), a lower initial occurrence of polyphenols in litter peat indirectly promotes microbial decomposition (16, 17).

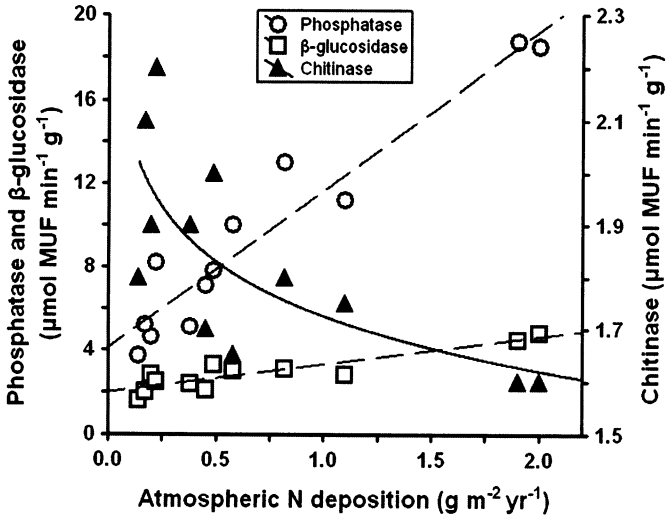

Fig. 2. Relationships between atmospheric N deposition and activity of phosphatase, β-glucosidase, and chitinase at the end of incubation. A positive relationship was found for phosphatase ($y = 4.1 + 7.5x$, $R^2 = 0.91$, $P < 0.01$; $n = 12$) and β-glucosidase ($y = 2.0 + 1.3x$, $R^2 = 0.81$, $P < 0.01$; $n = 12$), whereas a negative relationship was found for chitinase [$y = 1.7 - 0.1ln(x)$, $R^2 = 0.50$, $P = 0.01$; $n = 12$]. Values are means of three to six litter peat samples for each bog (SE was <15% of the corresponding mean value).

The direct and indirect effects of N availability on litter peat decomposition are connected through the activity of phenol oxidase, one of the few enzymes able to degrade polyphenols. The contrasting effects of N additions across different studies on phenol oxidase activity are primarily explained by the amount of lignin in plant litter (18–21), with species low in lignin showing a high activity and species high in lignin showing a low activity of phenol oxidase. Our finding of an increased activity of phenol oxidase with increasing N deposition (Fig. 3) is in accordance with the absence of lignin in *Sphagnum* plants (3), although these mosses contain peculiar, decay-resistant polyphenols (5, 22). An enhancement of phenol oxidase activity reduces polyphenol concentrations during litter peat decay (Fig. 3) and, indirectly, stimulates the activity of hydrolase enzymes (Fig. 2) via the phenol oxidase latch mechanism (15, 16) with an additional ultimate positive feedback on decomposition rate.

When nitrogen deposition is >1 $g \cdot m^{-2} \cdot yr^{-1}$, CO_2 emission shows a saturating trend (Fig. 1) that can be explained by an increasing P limitation on microbes (23, 24), as supported by the very steep increase in phosphatase activity (Fig. 2). In this case, the negative relationship between phosphatase and chitinase reflects a tradeoff in microbial resource allocation between two different enzymes when nutritional constraints change (12, 25), suggesting that, with high N deposition, P availability plays a primary role in controlling litter peat decay.

We also found a further potential C loss through an increased release of DOC from litter peat collected under higher N deposition (Fig. 4). We suggest that the increase of DOC concentration was a consequence of enhanced enzymatic activity, in particular of β-glucosidase (24, 26, 27), an extracellular enzyme involved in the release of C from organic matter. This biologically driven mechanism is supported by the significantly higher DOC concentration values at the end of the incubation compared with the correspondent initial amount of DOC released (Fig. 4), in accordance with increasing activity of β-glucosidase (Fig. 2). In addition, the absence of a relationship between DOC concentration and N deposition before the start

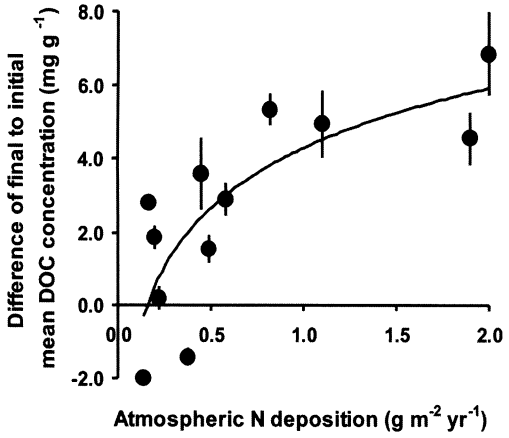

Fig. 4. N deposition and release of DOC. At the end of the incubation period the amount of DOC released was generally higher than the correspondent amount released at the beginning of incubation. Litter peat samples collected under higher atmospheric N deposition released a significantly greater amount of DOC compared with samples receiving a lower N input [$y = 4.3 + 2.4\ln(x)$, $R^2 = 0.61$, $P < 0.01$; $n = 12$]. Each value is the mean (± 1 SE) of three to six litter peat samples.

of the incubation ($r = 0.44$, $P = 0.16$; $n = 12$) and the correspondent positive correlation after 10 days of incubation ($r = 0.76, P < 0.01; n = 12$) support the primary role of enhanced metabolic activity of microbes in affecting the release of DOC. Furthermore, the role of enzymatic activity, i.e., a primary biological control over DOC production, is consistent with the negative correlation between molecular weight of dissolved organic matter and β-glucosidase activity ($r = -0.64$, $P = 0.03$; $n = 12$) as well as with the positive correlation between CO_2 emission and final DOC concentration ($r = 0.63$, $P = 0.03$; $n = 12$) (28, 29).

Increasing N availability can promote plant productivity in bogs in locations where N remains a limiting nutrient (30–32). However, *Sphagnum* productivity has only been shown to respond positively to atmospheric N deposition until a critical threshold of ≈1 g of $N \cdot m^{-2} \cdot yr^{-1}$, beyond which productivity is reported to decrease (30, 33–35). Moreover, increased N deposition may also give a competitive advantage to vascular plants (30, 36) whose litter is more easily decomposed (37). However, as *Sphagnum* remains the dominant peat-building component of European bogs, our findings indicate that high chronic N additions have the potential to promote peatland C release in gaseous and aqueous forms. The extent to which any future expansion of vascular plants might offset the reduced *Sphagnum* plant productivity and increased litter peat decomposition remains an important topic for future research. Such information would be a prerequisite for more precise prediction of the future peatland C storage capacity. Nevertheless, our results clearly highlight an urgent need for policy responses to combat global N eutrophication if we are to avoid the risk of a potential destabilization of these globally significant C stores.

Materials and Methods

Study Bogs. We selected 12 European bogs spanning a latitudinal gradient from ≈45° N to 63° N and a longitudinal gradient from ≈5° W to 17° E. In particular, one bog was selected in Norway ($n = 3$ peat samples), Finland ($n = 3$), Great Britain ($n = 3$), The Netherlands ($n = 6$), Switzerland ($n = 6$), and Slovenia ($n = 6$), whereas two bogs were selected in Sweden ($n = 3$ per bog), Czech Republic ($n = 6$ per bog) and Italy ($n = 6$ per bog). Data on atmospheric N deposition at each bog were obtained from national organizations responsible for precipitation monitoring (38) and refer to the 3 years preceding litter peat sampling. The natural gradient of chronic atmospheric N deposition spanned a range from 0.14 $g \cdot m^{-2} \cdot yr^{-1}$ (Sweden) to 2 $g \cdot m^{-2} \cdot yr^{-1}$ (The Netherlands). For a detailed description of study bogs and sampled habitats see Bragazza *et al.* (38).

Field Sampling. At each study bog, litter peat samples were collected in similar plant communities and microtopographic positions (hummocks and lawns) as previously described (35). The sampling was performed in areas with a dense cover of healthy *Sphagnum* plants and a very sparse, if any, cover of vascular plants. Hummocks had *Sphagnum fuscum* (Schimp.) Klinggr. and/or *Sphagnum capillifolium* (Ehrh.) Hedw. as dominant species, whereas lawns were mostly dominated by *Sphagnum magellanicum* Brid. A total of 30 hummocks and 27 lawns were available (see above). Because decomposition of *Sphagnum* litter is a continuous process, we chose recently formed *Sphagnum* litter (hereafter called "litter peat") at ≈2–4 cm below the bog surface in accordance with previous studies (39, 40).

Litter Peat Incubation and CO_2 Measurements. Remnants of vascular plants were removed from litter peat samples before incubation. A microbial inoculum was prepared by mixing 0.5 kg of fresh *Sphagnum*-dominated litter peat with 1.5 liters of deionized water. After 30 min of stirring, the slurry was filtered through a coarse glass filter and used to inoculate the collected litter peat samples. Approximately 0.1 g of air-dried litter peat was aerobically incubated with 8 ml of slurry in glass vials in darkness at a constant temperature of 12°C and shaken frequently. After 4 and 10 days, the glass vials were sealed, and evolved gases were allowed to accumulate for 1 h. A gas sample was then collected from each glass vial by using a gas-tight syringe, and evolved CO_2 concentrations were analyzed on a gas chromatograph (AMS Model 92, Analytical Measuring Systems, Cambridge, U.K.). Concentrations were then converted to CO_2 production per initial gram of litter peat (dry weight; see below) and per hour of incubation, taking into account the CO_2 concentration produced by the slurry used for the inoculum (six replicates).

Chemical Analyses. Total concentration of N and P in litter peat samples was determined colorimetrically on a flow-injection autoanalyzer (FlowSys, Systea, Anagni, Italy) after acidic digestion by following the Kjeldahl procedure. Litter peat C concentrations were determined on an elemental analyzer (EA 1110, Carlo Erba, Milan, Italy). Litter peat subsamples were dried at 40°C for 24 h to convert all concentrations to oven dry weight. DOC concentrations were determined as the difference between total C and inorganic C by using a total C analyzer (Shimadzu 5000, Kyoto, Japan). Initial DOC concentration released from each litter peat sample was determined after stirring ≈0.1 g of air-dried peat in 8 ml of distilled water for 4 h. After 10 days of incubation, final DOC concentrations were determined in the extract formed by the slurry plus the litter peat. Before DOC and polyphenol analyses, all samples were filtered through a 0.45-μm glass filter. DOC concentration present in distilled water and in the slurry was taken into account in the calculation. The initial and final concentrations of polyphenols were determined in the same solution used for DOC by following the Folin–Ciocalteu procedure (26). Polyphenol concentrations in distilled water and in the slurry were taken into account in the calculation. Both DOC and polyphenols were expressed per initial gram of litter peat (dry weight).

Enzymatic Assays. After 10 days of incubation, each litter peat sample was assayed for the activity of hydrolases β-glucosidase, chitinase [*N*-acetylglucosaminidase (NAGase)], and

phosphatase, as well as for the activity of oxidative phenol oxidase (15). The activity of hydrolase enzymes was detected for each litter peat sample by adding 4-methylumbelliferyl (MUF)-β-D-glucoside for the activity of β-glucosidase, 4-MUF-*N*-acetyl-β-D-glucosaminide for the activity of chitinase, and 200 μM 4-MUF-phosphate for the activity of phosphatase to an aliquot of extract (peat plus slurry). After incubation (1 h for β-glucosidase and chitinase and 45 min for phosphatase) at 12°C, the fluorescence of the supernatant was measured immediately on a microplate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany) at 450-nm emission and 330-nm excitation wavelength. To quantify product release and account for quenching effects, a set of standards was prepared according to the above procedure (methylumbelliferone replaced the MUF substrates). Enzyme activities were expressed as μmol of substrate (MUF) converted per minute and per gram (dry weight) of litter peat. The activity of phenol oxidase was determined spectrophotometrically by using 10 mM L-dopa (dihydroxyphenylalanine) solution as substrate. The activity of phenol oxidase was expressed as μmol of 2,3-dihydroindole-5,6-quinone-2-carboxylate (dicq) $h^{-1} \cdot g^{-1}$ litter peat (dry weight).

Spectroscopic Measurements. The quotient of wavelength absorption at 365 nm and 250 nm was used as the indication of molecular weight of dissolved organic matter (41).

Statistical Analyses. A multiple backward regression of CO_2 emission (after 4 days) against N deposition, latitude, elevation of the bog, mean annual temperature, and annual precipitation was initially performed. N deposition was identified as the only significant predictor variable ($F_{1,10} = 14.9, P = 0.003, R^2_{adjusted} = 0.56$; standardized regression coefficient for N deposition = $0.26, P < 0.01$) accounting for differences in litter decomposition compared with climatic conditions and geographical position. All statistical analyses were performed after averaging the hummock and lawn data of the same bog. Pearson correlations (r) and univariate regressions (R^2) were used.

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